The Signalling Function Of Eyespan In Stalk-Eyed Flies (Diptera: Diopsidae)

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ABSTRACT:

Genetic models of the handicap theory of sexual selection propose that ornaments signal heritable male quality, so females mating with the most ornamented males acquire fitness benefits for their offspring. Male ornaments are predicted to have co-evolved with female preference to be larger, and so more costly. The key prediction made by the handicap hypothesis is that male sexual traits have evolved heightened condition dependence, a result of the higher differential cost of ornaments relative to other traits. I investigated evidence for condition-dependent sexual ornaments and found little support from well-designed experiments. Most studies had neglected to 1) compare condition dependence in sexual traits with suitable non-sexual controls, 2) adequately account for body size variation, and 3) assess individuals under a range of stresses representative of those experienced in nature. There was also a dearth of experimental studies exploring the genetic basis of condition dependence.

I used experiments with stalk-eyed flies to examine predictions made by condition-dependent handicap models of sexual selection. *Cyrtodiopsis dalmanni* is highly sexually dimorphic for eyespan, and females exhibit strong mating preferences for males with large eyespans. Condition was varied experimentally by manipulating larval food availability. I found that male eyespan was more sensitive to changes in condition than female eyespan and other non-sexual traits. Male eyespan also showed a great increase in standardized phenotypic variance under stress, unlike non-sexual traits. These patterns persisted before and after controlling for body size. In contrast, there was no heightened condition dependence of male eyespan in *Sphyracephala*

beccarri, a species without female mate choice for exaggerated male eyespan and only minor sex differences in eyespan.

The genetic basis of ornament condition dependence was investigated in *C. dalmanni* by comparing the performance of distinct genotypes (inbred lines) along a gradient of environmental stress. Lines that produced a large ornament in one environment tended to do so in others. Stress also amplified these differences between genotypes leading to an increase in the genetic variance of the male ornament. Such patterns were less marked in non-sexual traits, and persisted after controlling for size. I looked for positive correlations between ornaments and viability by assessing the genetic correlations between male eyespan expression and four components of fitness (male fertility, female fecundity, and male and female longevity). I found no evidence that females obtain genetic benefits, other than male attractiveness, for their offspring by mating with well-ornamented males. However, body size-corrected male eyespan was negatively correlated with female longevity. This was unexpected and does not provide support for "good genes" benefits of sexual selection. Possible reasons for such findings (or lack thereof) are discussed.

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ACKNOWLEDGEMENTS

I am indebted to my supervisors, Professor Andrew Pomiankowski and Dr Kevin Fowler for help, advice and constructive criticism throughout the tenure of my studentship and the production of this thesis. Thanks also to past and present members of the Stalkie Empire at UCL (Rick Baker, Martin Carr, Tracey Chapman, Claire Grant, Penny Haddrill, Imogen Hurley, Ken Kraaijeveld, Ellen McConnell, Piedad Reguera,), and in particular to Matt Denniff and Dave Rogers. Janne Kotiaho (also Mats Bjorklund, Mike Ritchie, Jens Rolff, and four anonymous reviewers) provided helpful and insightful comments on publications derived from work presented here. The Natural Environment Research Council (NERC) is acknowledged for providing funding, and the Department of Biology, UCL awarded me with scholarships throughout my time in London. Finally, special thanks are extended to my family and to Rebecca Tollervey for providing constant encouragement and support.

1

General Introduction

1.1 OVERVIEW

This introduction serves two purposes. First, it introduces elaborate male secondary sexual ornaments as problems of evolution by natural selection, and sets such difficulties in a historical context. I discuss the various hypotheses evoked to account for their presence in nature, and describe the two main theoretical resolutions that have been proposed. I concentrate on so-called "condition-dependent" or "strategic" handicap models of sexual ornament evolution, and only on those that employ "good genes" as the driving force; sexual selection driven by direct benefits that enhance female fitness (as opposed to offspring fitness) is not discussed. I then briefly review one of the main objections raised against handicap models (the maintenance of genetic variance in fitness), and comment on recent advances concerning the interplay between natural and sexual selection. This first section is primarily an overview of the theory, as empirical data are discussed in the body of this thesis. The second part of this introduction acquaints the reader with the rest of the dissertation by outlining the aims and content of subsequent chapters. It starts by introducing stalk-eyed flies (Diptera: Diopsidae) as model organisms, and provides details on the natural history and evolution of Diopsids.

1.2 THE EVOLUTION OF SEXUAL ORNAMENTS: A HISTORICAL PERSPECTIVE

Darwin's (1859) theory of evolution by natural selection provides a clear and conceptually simple mechanism that accounts for the existence of traits that confer a fitness advantage. It cannot however, explain the obviously maladaptive secondary sexual characteristics of males from many species. If these elaborate traits, such as bright colouration or exaggerated morphologies, song or other behavioural displays, or horns and weapons, really enhanced survival then we would expect to see them in females as well. Neither can they readily be explained through any benefit to kin, as with other sex-limited traits (e.g. placentas, mammary glands, ovipositors). Darwin concluded that a second evolutionary force, sexual selection, was responsible for the exaggeration of male sexual traits, which "depends not on a struggle for existence, but on a struggle between males for possession of females; the result is not death to the unsuccessful competitor, but few or no offspring" (Darwin 1859 p. 136).

Darwin (1871 II p. 257-258) held the view that male "weapons of offence", "courage" and "pugnacity" evolved through direct competition between males for access to females, and that male ornaments "serv[e] *only* to allure or excite ... female[s]" and are used in indirect competition between males (emphasis mine). His belief in the existence of female preference and its importance in the evolution of male traits was particularly strong; "I see no good reason to doubt that female[s] ... , by selecting, ..., the most melodious or beautiful males, according to their standard of beauty, might produce a marked effect", and will "have added to [the male's] beauty" (Darwin 1859 p. 137, 1871 II p. 259, respectively). Whilst Darwin clearly alludes to the possibility

that females from each species have their own preference or "standard of beauty" for mates, he does not provide an evolutionary explanation for why they should.

Criticism of Darwin's (1859, 1871) theory of sexual selection came from Wallace, among others (reviewed in Pomainkowski 1988; Andersson 1994). Although Wallace accepted that sex-limited male weapons evolved through mutual combats, he argued that this was just an extension of natural selection acting on vigour and fighting ability. He also explained the presence of elaborate male traits using natural selection-based processes such as warning colouration, mimicry and recognition, and vehemently rejected the notion that female choice was directly involved in the evolution of male sexual traits (Wallace 1889). However, Wallace's conviction that ornaments were the result of natural selection did lead him to concede that female choice may work in concert with natural selection if it acts on overall male quality or those traits that indicate male vigour indirectly.

Fisher (1915) was the first to directly address the problem of why female mate preferences had evolved in the first place. He promoted an adaptive explanation for female choice by noting that, "the tastes of organisms, like their organs and faculties, must be regarded as products of evolutionary change, governed by the relative advantage which such tastes may confer"(Fisher 1930 p. 136). Thus Fisher argued that female mate preference can evolve and will be adaptive if females gain fitness advantages from mating with favoured males.

The insights of Darwin, Wallace and Fisher provide important foundations for contemporary theories of male ornament evolution because they suggest that: 1) female preference for male characteristics can lead to such traits becoming so exaggerated that they impinge on male survival, 2) females may exhibit choice for

traits that indirectly indicate male quality, and that, 3) female preferences themselves evolve because the consequences of choice can alter female fitness.

1.3 MODELS OF SEXUAL SELECTION THROUGH FEMALE CHOICE

1.3.1 Fisher's "Runaway" Process

Fisher (1915, 1930) argued that female preferences, like male ornaments, are the products of selection. He proposed that preference for certain male traits will become established if those male traits confer a natural selection advantage; under such circumstances preference would spread because the offspring of discriminating females would have greater fitness. However, his key observation was that as a result of non-random mating, female preference alleles would become genetically coupled with alleles for the preferred trait. Fisher (1930 p. 136) noted that this genetic covariance between choice and ornament would have "remarkable consequences" for the evolution of both characters, as it alters the relative mating success of males, and with it the relative advantage of preferring such males as mates. Choosy females would produce sons who possessed the preferred trait, and the mating advantage of these preferred males will not only lead to a selected increase in the male trait, but also to the spread of the preference genes that are genetically linked to it. Fisher concluded (1930 p. 137) that male ornaments and preferences for them "must thus advance together, and so long as the process is unchecked by severe counter selection, will advance with ever-increasing speed". He went on to comment that "it is easy to see that the speed of development will be proportional to the development already

obtained ... [and that] the potentiality of a runaway process ... must, unless checked, produce great effects" (*ibid.*). This secondary "runaway process" was solely the result of sexual selection, and would cause exaggeration of male traits to exceed that favoured by natural selection alone. It would only be arrested when counter-selection against the ornament (though the viability cost of possessing a large ornament) out-weighed the mating advantage associated with it.

What Fisher characteristically found "easy to see" was not formalised for a further fifty years. Lande (1981) and Kirkpatrick (1982) were the first to confirm that Fisher's process was a viable model for the evolution of male ornaments through female preferences (but see O' Donald 1962, 1967). They both found that stable outcomes of ornament and preference co-evolution occurred not as distinct points, but as a line of equilibria. For each value of the preference trait there is a stable level of male ornament exaggeration where the mating advantage of preferred males is exactly balanced by the reduced survival that results from having a large ornament. Consequently, there is no selective pressure on female mate preferences at equilibrium. If the system is dislodged from the line of equilibria, then the outcome is dependent on the slope of the evolutionary trajectory of preference and ornament. If the evolutionary trajectory is less than the slope of the line of equilibria, then the system will evolve back towards a new internal equilibrium. However, if the evolutionary trajectory is greater than the slope of the line of equilibria, for instance if females are sufficiently critical in their choice of mate or if natural selection against the male ornament declines, then deviations are unstable and will lead to the evolution of increasingly larger traits and stronger preferences as predicted by Fisher (Lande 1981).

These models of runaway evolution are based on the assumption that female fitness is maximised at equilibrium. Fisher's process cannot produce stable evolution of exaggerated ornaments if fitness costs are incurred as a result of mate choice (Kirkpatrick 1985; Pomiankowski 1987a). Costly preference destroys the line of equilibria and the population evolves to a single equilibrium where female fitness is maximised (i.e. zero preference, zero ornamentation; Pomiankowski *et al.* 1991). Pomiankowski *et al.* (1991) have shown however, that the Fisher process can lead to the stable exaggeration of male traits in spite of costly female choice if there is a deleterious mutation bias in ornaments. Biased mutation will result in an excess of unattractive males, who although subject to weaker natural selection, have reduced fitness because they incur a mating disadvantage from being poorly ornamented. Thus choosing attractive males can still be beneficial even when costly, and populations can evolve to a single equilibrium where the costs of mate preference are exactly offset by the Fisherian benefit of having more attractive sons.

1.3.2 Zahavi's "Handicap Principle"

Whilst most attention regarding Fisher's theory of sexual selection has focused on the runaway process, he believed that his two-stage model (i.e. natural followed by sexual selection) for the evolution of ornaments and mate choice would work "so long as the sons of [females] exercising the preference ... have any advantage over the sons of other [females], whether this be due to the first or the second cause" (Fisher 1930 p. 136). The first cause, a natural selection advantage, was clearly viewed by Fisher as important, but he took the cost of ornaments to be just a by-product of their exaggeration.

Zahavi (1975) favoured an alternative theory, suggesting that ornaments evolved precisely because they were costly. Rather than females preferring well-ornamented males solely for the benefits associated with attractive sons, he advised that female preference existed in order that they may mate with males of high viability. Following Maynard Smith (1987 p. 12), I use the term "viability" to mean components of fitness other than mating success. Zahavi (1975, 1977) proposed costly male ornaments acted as a "handicap", whereby only males of high viability are able to survive with the extra burden of a large deleterious ornamental trait; females exhibiting preference for handicapped males will have offspring of higher than average viability and so be favoured by selection (assuming that viability is heritable; see below).

The "handicap" principle received much early criticism principally because it did not drastically alter the conditions for the spread of the handicapping ornament or preference genes beyond those predicted by the Fisher process (reviewed in Maynard Smith 1985). In addition, when Fisher's runaway was prevented (in models using monogamous populations) the handicap principle was judged to be ineffective (Andersson 1982). However, Pomiankowski's (1987b) formalisation proved that handicaps, and preferences for them, can and do spread, but only in conjunction with the Fisher process. In a three-locus model, he showed that the line of equilibria found in runaway models is abolished when the handicap principle operates. Any covariance between viability genes and the ornament destabilises the population and causes the handicap to spread to fixation and form a new equilibrium.

Three different types of handicap have been proposed: Zahavi's handicap, revealing handicaps and condition-dependent handicaps (reviewed in Pomiankowski 1988; Andersson 1994). I will only consider the condition-dependent model as this

has proven to be a more potent force than other classes of handicap, and has tended to dominate sexual selection theory. Condition-dependent handicaps are assumed to be expressed in proportion to the overall quality or condition of their bearer (Zahavi 1977; Kodric-Brown & Brown 1984; Andersson 1986; Zeh & Zeh 1988; Pomiankowski & Møller 1995; Rowe & Houle 1996). Condition is defined as showing strong covariance with general viability, such that higher values confer greater fitness. Iwasa and Pomiankowski's (1994; see also *et al.* 1991) quantitative genetic model will be used to describe the major requisites for the evolution of condition-dependent handicaps.

Iwasa and Pomiankowski examined the evolution of costly male ornaments and costly female choice in a model with four traits, t, t', p and v. All traits are polygenic, and for simplicity only additive genetic effects were studied. Turning first to males, the realised ornament size (s) follows a simple linear model and is dependent on three factors, the value of genes for the male trait *per se* (t), male viability (v) and a condition dependence parameter (t'). Specifically

$$s = t + t'v \,. \qquad (1)$$

The value of t' reflects the relationship between ornament size and general viability. If ornaments are wholly Fisherian traits then their expression is independent of viability and $t' = 0$. If $t' > 0$ then ornament size is an increasing function of male viability. t and t' are sex-limited in males, whilst v is expressed equally in both sexes.

Male fitness is determined by two components: sexual and natural selection. Male mating success increases (exponentially) at a rate proportional to the strength of the average female preference trait, $\bar{p}$. Preference genes are only expressed in females

and females mate at random if $p = 0$. Positive values of p result in females preferring males with larger than average ornaments ($s - \bar{s} > 0$), so well-ornamented males gain a sexual selection advantage when $\bar{p} > 0$. The naturally selected component of male fitness comprises two elements. The first is due to the direct effect of male viability, where fitness increases as a function of v. The second arises through the costs associated with ornament size,

$$\text{cost} = \frac{c}{1 + kv} s^2 . \qquad (2)$$

c and k are constants, and costs increase as the male trait deviates from its naturally selected optimum (for simplicity, $s_{opt} = 0$). Survival chances decline symmetrically around this point. A crucial assumption of the handicap hypothesis is that survival chances for a given ornament size vary with male viability (v). The cost differential, k, mediates how male viability affects the deleterious consequences of ornamentation. If $k = 0$, then male viability has no effect on survival, whereas when $k > 0$, poor quality males (i.e. low v) pay higher costs for a given ornament size than males with higher v.

As with males, female fitness is an increasing function of the naturally selected viability trait (v). However, mate choice is assumed to be costly. Random mating ($p = 0$) has no cost, but discriminating females ($p > 0$) incur reductions in fitness that scale in (exponential) proportion to the strength of preference. Unlike the cost of male ornamentation, it is assumed that the viability trait does not influence the costs of choice.

Iwasa and Pomiankowski (*et al.* 1991, 1994) found that two conditions were essential for the evolution of costly mate choice under the handicap principle. First,

the viability trait must be subject to a deteriorating force (such as biased deleterious mutation) that maintains genetic variation in fitness, and second, ornaments must be expressed in a condition-dependent fashion ($t' > 0$). Iwasa and Pomiankowski (1994) also demonstrate that $t' \propto k$; condition dependence only evolves when the differential cost of ornament expression causes lower quality males to pay higher survival costs for larger sexual ornaments (i.e. when $k > 0$). Thus ornaments only become condition-dependent when the costs of exaggeration impinge on survival. These requirements ensure that females are able to evaluate male ornaments and gain heritable fitness benefits for their offspring in spite of any costs to their own fitness that they incur from doing so. At equilibrium (when $\Delta p = 0$) the costs of mate preference are exactly balanced by the benefits accrued through the increased viability of offspring.

Most successful models of the handicap theory have worked because they show that handicaps can evolve as stable evolutionary outcomes in conjunction with the Fisher's (1915, 1930) self-reinforcing process. However, Grafen (1990a; see also 1990b) addressed the question of whether the handicap principle can act as a "stand-alone" force in sexual selection. He constructed a game theory model (Maynard Smith 1982) to investigate handicap evolution where Fisher's process plays no role.

Grafen assumed that males vary in quality, q, and that it pays females to mate with high quality males. Females cannot observe q directly. However, males can give a perceptible advertisement of quality, a, that is costly to produce. The function relating quality to the level of advertisement is $A(q)$. Whilst a male cannot alter his quality, he can alter his level of advertising. The probability of male survival is denoted by $\alpha(q,a)$, and is an increasing function of quality and a decreasing function of advertisement level. As with previous condition-dependent handicap models, the

survival cost of a given level of advertising is smaller for higher quality males, such that

$$\frac{\alpha(q_2,a_2)}{\alpha(q_1,a_1)} < \frac{\alpha(q_2,a_2)}{\alpha(q_2,a_1)} \qquad (3)$$

if $a_1 < a_2$ and $q_1 < q_2$. The rule which decides whether a female will mate with a male is given by $D(a,t)$. The probability of mating increases with a, whilst t denotes the time in a finite breeding season at which the mating opportunity arises; in short t is a measure of female choosiness or willingness to mate, and is associated with the potential cost of remaining unmated at the end of the season.

The evolutionary variables in Grafen's model were therefore $A(q)$ and $D(a,t)$, representing male and female strategies respectively. He derived a pair of functions, $A^*(q)$ and $D^*(a,t)$, which were evolutionarily stable at equilibrium. Under such conditions, no male strategy other than A^* has greater fitness in a population using A^* and D^* rules, and no alternative female strategy can do better than D^* in a population of A^*D^* individuals. Thus a population consisting of A^*D^* strategies is uninvasible by mutants using different rules of advertisement or choice.

Grafen (1990a) found that a signalling equilibrium existed if females were able to successfully infer male quality from his advertisement. This required that advertisement was costly, and that the cost varied depending on male quality such that a given signal was more costly for a male of low quality (i.e. if the inequality in (3) held). Thus he reached the same conclusions as other successful models of the handicap theory. However, what made Grafen's model unique was that he modelled the entire system on one locus in a sexual haploid population. So a single locus specified both the male advertising rule, $A(q)$, and the female preference rule, $D(a,t)$,

although advertisement and choice were sex-limited. This ensured that Fisher's process played no role in the outcome, as independent genetic variation in each of the traits, as well as covariance between them, is needed for runaway.

The handicap process works if 1) differential costs maintain honesty in signalling, and 2), honesty ensures that females can infer male quality accurately and reliably from the size of the ornamental trait. Grafen's (1990a,b) model assumes that all males employ the same signalling strategy at equilibrium (i.e. condition dependence is constant with no variance). In reality however, signallers are bound to differ and male ornament size will tend to over-estimate quality in some individuals and under-estimate it in others. "Deception is the inevitable result" (Johnstone & Grafen 1993 p. 762). The extent to which such cheater males prosper depends critically on their frequency in the population. If high, then females will pay the cost of choice but will not reap the viability benefits for their offspring, so selection will favour a reduction in female preference. However, if the frequency of cheaters is low, then the females will gain benefits from choice on most occasions, and ornaments need therefore only be honest "on average" (Johnstone & Grafen 1993). An additional problem concerns imperfection in the evaluation of male ornament size by females. Incorporation of perceptual error by females into handicap theory increases the biological realism of models and allows assessment of both the "strategic" and "efficacy" components of signal design (Guilford & Dawkins 1991; Johnstone & Grafen 1992; Johnstone 1994; reviewed in Johnstone 1997). Using an ESS approach, Johnstone and Grafen (1992) showed that perceptual error is unlikely to disrupt the stability of the handicap equilibrium so long as the average inferred quality of a male is an increasing function of his true quality. Under these conditions a high quality male that advertises at a high level is more likely to be perceived by females as

advertising at a high level. This will preserve the relationship between the female's perception of male ornament size and true male quality.

1.4 THE MAINTENANCE OF GENETIC VARIATION IN FITNESS

Along with ornament condition dependence, an essential pre-requisite of all indirect (genetic) models of the handicap principle is the presence of additive genetic variance in fitness. This ensures that females can offset the costs of mate choice through increased offspring viability. Much of the early criticism levelled at the handicap theory arose because population genetics theory predicts that populations close to or at equilibrium have little or no additive genetic variance in fitness (Maynard Smith 1978; Charlesworth 1987); directional selection is expected to deplete genetic variation in fitness as favoured alleles will quickly spread to fixation. Since Fisher's 'Fundamental Theorem of Natural Selection' states "the rate of increase in fitness of any organism at any time is equal to its genetic variance in fitness at that time" (Fisher 1930 p.35; see also Price 1972; Crow 2002), then the required positive parent-on-offspring correlation for fitness is unlikely at equilibrium. Empirical support for this potentially fatal problem comes from the finding that the heritability of a trait (the ratio of additive genetic variance (V_A) to total phenotypic variance (V_P)) is negatively correlated with its contribution to fitness; traits closely related to, or approximating, fitness have heritabilities close to zero (Gustaffson 1986; Mousseau & Roff 1987; Roff & Mousseau 1987). The low heritabilities in such studies have been taken as evidence for depleted levels of additive genetic variance in fitness (components). This has given rise to the 'lek paradox' of sexual selection: why

do females, who receive only genes during mating, continue to discriminate between males if there are so few genetic benefits of choice (Borgia 1979; Taylor & Williams 1982; Kirkpatrick & Ryan 1991)?

However, Houle (1992) observed that a low heritability may reflect low levels of V_A, or it may result from high levels of residual variance that elevates V_P. Houle (1992) showed that standardising V_A by the trait mean ($\overline{X}$) rather than by V_P, and deriving coefficients of additive genetic variation ($CV_A = 100\sqrt{V_A}/\overline{X}$) produced a metric that was more suitable for comparing the additive genetic variance of traits. Life-history traits were found to have significantly higher CV_As than other traits, suggesting that fitness and its components exhibit large amounts of additive genetic variance that may sustain eugenic sexual selection. It is also noteworthy that Pomiankowski and Møller (1995) found that male ornaments had high CV_As relative to non-sexual traits.

How is genetic variance in fitness maintained? This topic has been reviewed extensively elsewhere (e.g. Falconer & Mackay 1996; Roff 1997; Maynard Smith 1998), and I only comment here on the fundamental mechanism for variance generation (mutation), and one other that has received considerable attention within sphere of sexual selection (host-parasite coevolution). It is important to remember that these mechanisms are not mutually exclusive, and all that is required for handicap models to work is that the viability trait suffers a constant loss of adaptation each generation through a continually deteriorating selective environment.

As mutations are the ultimate source of all genetic novelty, then variance in fitness may persist through a balance struck between the variance introduced through mutation on one hand, and the variance depleted by selection on the other (Roff 1997). Whilst the per locus mutation rate is likely to be low (as a result of DNA repair

and other preservative methods), the number of mutable genes that affect viability or fitness is likely to be large, and may potentially include all loci in the genome (Houle 1991). Rice (1988; see also Kondrashov 1988a; Burt 1995) has estimated that deleterious mutations in the genome of *Drosophila melanogaster* may reduce total fitness by almost 20%, suggesting that there is substantial variation in the viability of natural populations.

The other predominant mechanism for maintaining high levels of variance in fitness is that of host-parasite coevolution. Hamilton and others (Hamilton & Zuk 1982; Eshel & Hamilton 1984) have shown that parasites can be potent agitators of evolutionary equilibria, preventing the complete depletion of genetic variance in the fitness of their hosts. For instance, Eshel and Hamilton (1984) modelled viability as a locus that confers parasite resistance, and demonstrated that cyclical evolution between host and parasite(s) causes selection coefficients for each host viability genotype to fluctuate over time. Assuming that gene frequencies did not reach fixation, they observed persistent variance in fitness and the high parent-on-offspring correlations of viability that are required by genetic models of the handicap principle.

1.5 THE INTERPLAY BETWEEN NATURAL AND SEXUAL SELECTION

A number of recent studies have suggested that the condition dependence of ornaments that evolves via the handicap principle has far reaching consequences outside the arena of sexual selection. Lorch *et al.* (2003) have shown that the evolution of condition-dependent ornaments allows both natural and sexual selection to work in tandem on the viability trait. The combination of these two selective

pressures leads to elevated equilibrium mean fitness, and increases in the speed of attainment of equilibrium in fluctuating environments (relative to populations where condition dependence of ornaments is absent). Thus sexual selection for condition-dependent ornaments can enhance the rate of adaptation (Lorch *et al.* 2003).

Condition-dependent ornaments have also been implicated in the evolution of sex itself. Sexual reproduction is problematic because of the two-fold fitness cost it entails relative to asexual reproduction (Maynard Smith 1978). In order for sex to be evolutionarily viable it must overcome this fitness deficit. One of the leading theories for the maintenance of sex proposes that sexual reproduction reduces the genetic load in a population (Kondrashov 1988b). This requires that synergistic interactions between deleterious mutations cause a greater than linear decline in fitness (Kondrashov 1988b), and it is presently unclear whether such epistasis occurs.

Recently, differential male mating success through sexual selection has been shown to lead to large reductions in the mutation load of a sexual population if sexual selection causes deleterious mutations to be more harmful in males than females (Agrawal 2001; Siller 2001). These conditions are readily met when considering the costs of male ornament expression. Handicapping ornaments, by definition, depress mean male viability. The high differential cost of ornaments with respect to the number of deleterious mutations in a male's genome (i.e. viability) means that female choice based on such ornaments will tend to reduce the number of mutations entering the next generation and thus purge mutations from the population. The reduced fitness of handicapped males is of no consequence in questions concerning the evolution of sex, as they are already included in the two-fold cost. However, at equilibrium the frequency of deleterious mutations will be lower in sexual females than asexuals, thus providing a relative advantage to sex. So "extravagant ornamental waste [in males]

actually reduces the cost of sex ... [and] ... may have had far reaching consequences for the evolution of complex life" (Siller 2001 p. 692).

The preceding discussions were presented to clarify the theoretical arguments surrounding the evolution of exaggerated male ornaments, and costly mate preferences for them. They also serve to highlight the importance of condition dependence in the evolution of sexual signals. This thesis is concerned primarily with that latter point, that male ornaments have evolved condition-dependent expression in order to signal "good genes" in their bearer. Much of the work reported in this volume is experimental, using stalk-eyed flies (Diptera: Diopsidae) to assess predictions made by the handicap hypothesis. What follows is a brief review of the natural history of the Diopsidae and some remarks about their evolution, through both natural and sexual selection, which are pertinent to the rest of the dissertation. A brief résumé of each chapter is then given to prime the reader of what lies ahead.

1.6 STALK-EYED FLIES: MODEL ORGANISMS IN SEXUAL SELECTION

Stalk-eyed flies (Diptera: Diopsidae) are increasingly becoming viewed as important models for testing predictions made by sexual selection theory (Andersson 1994; Wilkinson 2001; Wilkinson & Dodson 1997; Maynard Smith & Harper 2003). In this section I briefly review their evolution and natural history, and discuss the empirical findings that relate to sexual selection.

Diopsids are characterised by hypercephaly, the elongation of the head capsule into long stalks onto which the eyes and antennae are laterally displaced

(Baker *et al.* 2001). Since their discovery by Linnaeus (1775; reviewed in Shillito 1974), over 150 species have been documented (Feijen 1989) with the true number probably nearing 300 (Wilkinson & Dodson 1997). Whilst hypercephaly has arisen in several Dipteran families (Grimaldi & Fenster 1989; Wilkinson & Dodson 1997), Diopsids are unique in that both sexes of all species possess stalked eyes (Baker *et al.* 2001). Most stalk-eyed flies are found in the tropics of South East Asia and Africa, although the genus *Sphyracephala* is more widespread, with representatives in North America and Europe (Feijen 1989; Papp *et al.* 1997; Wilkinson & Dodson 1997).

Adult Diopsids typically live off fungi, mould and decaying leaf litter (Burkhardt & de la Motte 1983; Feijen 1989; Wilkinson & Dodson 1997; but see Paulovics 1998). Adults are capable of a long lifespan, and many species can be kept alive in captivity for over 6 months (S. Cotton *personal observations*). Similar longevity has been reported in wild populations (Wilkinson & Reillo 1994; Wilkinson & Dodson 1997). Larvae are saprophagous, and feed on decaying vegetation (de la Motte & Burkhardt 1983; Feijen 1989; Wilkinson & Dodson 1997). As larval food deteriorates, pupation occurs quicker and at smaller larval sizes (Wilkinson & Dodson 1997; S. Cotton *unpublished data*).

Sexual dimorphism in eye-stalk length is apparent in many species, where males have larger eyespans than females (Wilkinson & Dodson 1997; Baker *et al.* 2001; Baker & Wilkinson 2001). Species with no sex differences in eyespan are known however, and monomorphic eye-stalks are believed to be plesiomorphic in the Diopsidae (Wilkinson & Dodson 1997; Baker *et al.* 2001; Baker & Wilkinson 2001). The initial evolution of laterally displaced eyes may be explained by a naturally selected advantage accrued through increased visual capacity. The number of ommatidia in each compound eye is an increasing function of eyespan (Burkhardt &

de la Motte 1983; de la Motte & Burkhardt 1983). Approximately 70% of the ommatidia contribute to a frontoventral binocular field of > 135° (Burkhardt & de la Motte 1983), and the resolution of the Diopsid eye is assumed to be on a par with that of other visually acute insects such as dragonflies (de la Motte & Burkhardt 1983). There has been surprising little experimental work on Diopsid vision, so for instance we still do not know whether they have the capacity for stereoscopic vision. However, this hypothesis seems unlikely to account for the occurrence of sexual differences in eyespan seen in the Diopsidae. For instance, if hyper-exaggeration of eyespan has a natural selection advantage, then one would expect the same selection pressures to act on the female trait and cause eyespan sexual dimorphism to recede. Sexual dimorphism could through natural selection if sexes inhabit different niches and experienced selection for larger or smaller eyespans as a result of ecological differentiation (e.g. Darwin 1871; Lande 1980; Statkin 1984). However, there is no evidence to support this claim in stalk-eyed flies, and it seems improbable, given that eyespan sexual dimorphism has evolved independently on at least four different occasions (Baker & Wilkinson 2001). Thus some sex-related propensity for eyespan exaggeration seems likely.

Behavioural observations and experimental studies on dimorphic members of the Malaysian genus *Cyrtodiopsis* have indicated that sexual selection is responsible for increased male eyespan. At night dimorphic species aggregate on roothairs which hang underneath stream banks (Burkhardt & de la Motte 1985; Wilkinson & Dodson 1997). Males fight for control of these roosting sites, and contests are usually won by the male with the largest eyespan (Burkhardt & de la Motte 1983, 1987; Lorch *et al.* 1993; see also Panhuis & Wilkinson 1999). Females prefer to alight and mate on roothairs controlled by males with the largest absolute and largest relative eyespan

(Burkhardt & de la Motte 1988; Burkhardt *et al.* 1994; Wilkinson & Reillo 1994; Hingle *et al.* 2001). As a result, females tend to exhibit clumped distributions whereas males tend to be overdispersed (Burkhardt & de la Motte 1987; Wilkinson & Reillo 1994; Wilkinson & Dodson 1997). These roosting threads are mating "hotspots", with over 90% of all copulations occurring during dawn the following morning (Lorch *et al.* 1993). Females usually mate at least once each day (Lorch *et al.* 1993), and males with large eyespans typically mate with all females in his harem, which can number up to 20 (Burkhardt *et al.* 1994). Male reproductive success in dimorphic Diopsids is therefore greatly skewed in favour of males with large eye-stalks (Burkhardt *et al.* 1994).

Using artificial selection, Wilkinson (1993; see also David *et al.* 2000) has shown that the ratio of male eyespan-to-body size (relative eyespan) has a strong genetic basis in the dimorphic species, *Cyrtodiopsis dalmanni.* He found that bi-directionally selected lines diverged significantly in relative eyespan after only 10 generations. Wilkinson concluded that the relationship between eyespan and body size can respond to sexual selection, and can thus explain the diversity of male eyespan morphology in the Diopsids. In the field, Wilkinson and Reillo (1994) found that the (sexual) selection intensity (Lande & Arnold 1983) on male relative eyespan (calculated as the covariance between the average harem size and the ratio of male relative eyespan over its standard deviation) was 0.69 standard deviation units per generation. This selection intensity of female choice was over half of that used by Wilkinson (1993) in his artificial selection study, and suggests that female choice in stalk-eyed flies is a strong evolutionary force. However, these conclusions are potentially confounded by the inability of ratios to completely control for body size (Packard & Boardman 1999; Cotton *et al.* 2004a). Ratios are only valid under the

narrow condition of true isometry, where trait allometries are linear and pass exactly through the origin. In *C. dalmanni*, eyespan allometries have negative intercepts and relative eyespan values tend to increase with body size. The findings of Wilkinson (1993) and Wilkinson and Reillo (1994) may therefore be partially attributed to selection on body size rather than eyespan.

Monomorphic species do not possess this suite of behaviours. In Malaysia, Burkhardt and de la Motte (1985) reported that all four dimorphic species, but only one of five monomorphic species, aggregate at dusk. In addition, monomorphic species do not show strong male-male competition (Burkhardt & de la Motte 1985; Panhuis & Wilkinson 1999) and females exhibit no significant preferences for male eyespan (Wilkinson & Dodson 1997; Wilkinson *et al.* 1998).

1.7 THE STRUCTURE OF THE THESIS

This thesis comprises five 'results' chapters followed by a discussion and recapitulation of the main findings. Chapter 2 is general, providing a critical re-assessment of the widely held view that male sexual ornaments are condition-dependent. The remaining chapters (3 to 6) report the findings of experimental investigations into the condition dependence, signalling function and genetics of male eyespan expression in stalk-eyed flies.

Chapter 2

The key prediction made by the handicap hypothesis is that male sexual traits have evolved heightened condition-dependent expression, as a result of the higher differential cost of ornaments relative to other non-ornamental traits (Iwasa & Pomiankowski 1994). I review published experimental data and find that there are few well-designed tests that support this critical hypothesis. Most studies have ignored the comparative nature of the question and have failed to compare the condition dependence of ornaments with that of suitable non-sexual traits. In addition, few experiments have investigated the importance of body size scaling on sexual trait expression, and most have failed to assay individuals in circumstances similar to those experienced in nature. Perhaps most worrying is the (almost complete) lack of exploration into the genetic basis of condition dependence, a feature that is so crucial for the handicap hypothesis. This chapter is used to define a good study of condition dependence, and such recommendations are used in subsequent chapters of the thesis. The greater part of Chapter 2 has been accepted for publication in *Proceedings of the Royal Society: Biological Sciences* (Cotton *et al.* 2004a), and as a chapter in the forthcoming book *Insect Evolutionary Ecology* (ed. J. Rolff) to be published by the *Royal Entomological Society.*

Chapter 3

Cyrtodiopsis dalmanni is highly sexually dimorphic for eyespan, and females exhibit strong mating preferences for males with large eyespans, both before and after controlling for body size. I experimentally investigated the condition dependence of

male eyespan by manipulating larval food availability, and found that it was more sensitive to changes in condition than the homologous character in females and other non-sexual traits. Male eyespan also showed a greater increase in variance with stress than other traits. These patterns persisted both before and after controlling for body size, suggesting that females can gain additional information about male phenotypic condition by assessment of eyespan than by other traits or body size. This chapter has been accepted for publication in *Evolution* (Cotton *et al.* 2004b).

Chapter 4

The experimental regime used in Chapter 3 was applied to *Sphyracephala beccarri*. Male eyespan is unexaggerated in *S. beccarri* and there is no evidence for female mate choice in this species. I tested the hypothesis that heightened condition dependence evolves only when ornaments become costly through exaggeration. Against this prediction, I found that male eyespan was more sensitive to changes in condition than a control trait in males. However, similar differences were also observed in females, and there were no differences between the sexes in the degree of heightened eyespan condition dependence. To complete the analysis I compared the response to stress in *S. beccarri* with that in *C. dalmanni* and demonstrated the positive association of heightened condition dependence with traits that have become exaggerated through sexual selection. The finding in *S. beccarri* that eyespan is a more sensitive indicator of condition than other traits even in an unexaggerated state, suggests that this may have acted as a pre-adaptation to its role in sexual signalling in other Diopsid species. These results are consistent with handicap theory and accord

with Fisher's original view of how sexual selection is initiated. This chapter has been accepted for publication in the *Journal of Evolutionary Biology* (Cotton *et al.* 2004c).

Chapter 5

Genetic models of the handicap principle assume a heritable basis to condition-dependent ornament expression. I extended my findings from Chapter 3 and tested this expectation in *C. dalmanni*. Inbreeding was used to create distinct genotypes, and the genetic basis of ornament condition dependence was investigated by comparing the performance of genotypes (inbred lines) along a gradient of environmental stress. I found that lines that produced a large ornament in one environment tended to do so in others. Importantly, stress inflated these differences between genotypes leading to an increase in the genetic variance of the male ornament. Such patterns were present to a much lesser extent in non-sexual traits, and persisted after controlling for size. These results strongly implicate "good genes" as the target and potential benefit of female choice in *C. dalmanni*.

Chapter 6

The morphological findings of Chapter 5 are used to investigate the between-line (genetic) correlations of male eyespan expression and four components of fitness (male fertility, female fecundity, and male and female longevity). This allowed me to examine any potential viability benefits the females may accrue from mating with attractive males. I found that male eyespan expression did not predict either

component of male fitness or female fecundity. However, after adjusting for body size, I found that male eyespan was negatively correlated with an estimate of female longevity. This was unexpected, and possible explanations for such a correlation are discussed.

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2

Do Sexual Ornaments Demonstrate Heightened Condition-Dependent Expression As Predicted By The Handicap Hypothesis?

2.1 ABSTRACT:

The handicap hypothesis of sexual selection predicts that sexual ornaments have evolved heightened condition-dependent expression. The prediction has only recently been subject to experimental investigation. Many of the experiments are of limited value as they fail on at least one of three basic design features: they 1) do not compare condition-dependence in sexual ornaments with suitable non-sexual trait controls, 2) neglect to adequately account for, or investigate the importance of, body size scaling and 3) typically consider only two stress treatments (low and extreme stress), rather than a range of stresses similar to those experienced in nature. There is also a dearth of experimental studies investigating the genetic basis of condition dependence. Despite the common claim that sexual ornaments are condition-dependent, the unexpected conclusion from this literature review is that there is little support from well-designed experiments.

2.2 INTRODUCTION

The handicap hypothesis of sexual selection has increasingly come to dominate discussions about the evolution of exaggerated sexual ornaments (Andersson 1994). It predicts that female preferences have evolved for exaggerated sexual ornaments which signal male genetic quality. Male sexual traits have co-evolved with female preference to be larger, and so more costly. One of the key predictions made by the handicap hypothesis is heightened condition-dependent expression of sexual ornaments (Pomiankowski 1987; Grafen 1990; Iwasa & Pomiankowski 1994). Males in good genetic condition are assumed to signal their quality through greater sexual trait size or more vigorous display. Males in worse condition are unable to do this because of the viability costs associated with such extravagance. The same logic applies when male quality varies due to environmental conditions and this affects fertility, parenting or some other directly important reproductive ability (Iwasa & Pomiankowski 1999). So sexual trait condition dependence can evolve to signal male genetic or environmental quality, or both.

It is widely assumed that condition dependence is a common feature of sexual traits. This is backed by previous reviews that list many examples of ornament size and courtship performance being positively correlated with measures of condition (Andersson 1994, Johnstone 1995). However, the bulk of the evidence reported in these reviews is just correlational. These are indicative of underlying causal relationship but correlation is not cause. There is a need for experimental studies to confirm that sexual ornaments have strong condition-dependent expression. In addition, surprisingly few studies in these older reviews report null or negative

relationships between ornaments and condition. This gives the impression of publication or study bias.

Over the last decade there has been an increasing use of experimental techniques to investigate condition dependence. In this chapter I assess the current state of experimental evidence and ask whether, like the correlational data, the experimental results stack up in support of the condition-dependent sexual trait hypothesis. A second aim of this review is to set out the criteria needed for carrying out a rigorous study. I show that improvements in understanding are limited by deficiencies in experimental design. This leads on to suggestions as to where further investigation needs to be directed.

2.3 HANDICAP THEORY

In this section I outline the theory behind condition-dependent signalling. Theoretical investigation of handicap models has been carried out using major gene (Pomiankowski 1987), game theory (Grafen 1990) and quantitative genetics models (Iwasa *et al.* 1991). Here I concentrate on a simple quantitative genetic treatment which sets out the major predictions of the handicap hypothesis (for more details see Iwasa *et al.* 1991, Iwasa & Pomiankowski 1994, 1999). Let s be the size of a male sexual ornament used by females in their mate choice,

$$s = t + t'v\,. \qquad (1)$$

Ornament size is determined by a condition-independent (t) and a condition-dependent ($t'v$) part. The model assumes a linear relationship between ornament size

and male additive genetic quality or condition (v). This is mediated by t', the degree of condition dependence. In the simplest case we ignore environmental variation assuming that it has a mean effect of zero on the sexual ornament. The basic assumption of the handicap hypothesis is that the cost of the ornament varies with male quality,

$$\text{cost} = \frac{c}{1 + kv} s^2 . \qquad (2)$$

For simplicity we set the natural selection optimum ornament size to $s = 0$, with survival chances declining symmetrically around this optimum. The rate of decline is set by the cost coefficient (c). Survival chances also depend on male quality (v). They decline more quickly for lower quality individuals at a rate that reflects the cost differential (k). If k is large, lower quality individuals (i.e. lower v) pay higher costs for larger sexual ornaments, whereas if $k = 0$, male quality has no affect on survival chances. Given that females prefer to mate with larger ornamented males, equilibrium conditions can be derived for the size of the male ornament (Iwasa & Pomiankowski 1994, 1999). Of relevance here, we can specify the relative condition dependence of the male ornament,

$$\frac{\bar{t}'}{\bar{t}} = k , \qquad (3)$$

where $\bar{t}$ and $\bar{t}'$ are the mean trait values. This shows that as the cost differential k increases, so does the mean condition dependence of the sexual ornament ($\bar{t}'$).

A previous interpretation of this result (Iwasa & Pomiankowski 1994) was that the cost differential was $k = 0$ under Fisher's runaway process (Fisher 1930), and there would therefore be no condition dependence (i.e. $\bar{t}' = 0$) in ornaments which did not signal good viability genes. This ignores the fact that most traits to some extent show condition-dependent expression. This is obvious when body size is closely associated with fitness, and many traits scale with body size. A better interpretation is that Fisher's runaway does not assume any greater cost differential in the ornament than seen in other traits, so there is no expectation of heightened condition dependence in sexual ornaments that only signal attractiveness genes. In contrast, the handicap hypothesis assumes greater differential costs, and so predicts heightened condition dependence in sexual ornaments compared to other traits.

In Eq(2) we assume that there is genetic variation in male quality. We can also consider environmental quality variation (e) between males. For instance, individuals may differ in the food and resources available during development or in adult life. Given that we expect that the cost equation has the same dependency on environmental quality as it does on genetic quality, we can simply substitute e for v in Eq(2), and then Eq(3) still holds. So the handicap hypothesis predicts that sexual ornament condition dependence should occur with environmental quality variation just as it does with genetic quality variation. This is important because environmental variation in quality may be high under natural conditions, or because the experimenter creates large differences in environmental conditions in order to investigate condition dependence.

2.4 WHAT IS CONDITION?

The theoretical position set out above views condition (or quality) as a trait closely related to viability (Iwasa *et al.* 1991; Iwasa & Pomiankowski 1994). Condition has the property that higher values confer greater fitness, and it can have genetic (v) and environmental (e) components (Iwasa & Pomiankowski 1999). This simplistic view does not specify how condition is related to quantities that can be easily measured by field and experimental biologists. Here I briefly discuss various approaches to this question.

Measures of condition have been used for a long time as general gauge of individual nutritional state, resources, energy reserves and health in a variety of biological contexts (Bolger & Connelly 1989; Rowe & Houle 1996). Typical indexes attempt to control for body size by taking simple ratios (mass divided by body sized cubed), slope-adjusted ratios (slope of log(body mass) on log(body size)), or residuals (Jakob *et al.* 1996). All of these indexes have been criticised, largely because the true relationship between the two variables is unknown *a priori*, and each method introduces assumptions which may not be biologically or statistically justified (see Jakob *et al.* 1996; Kotiaho 1999; Packard & Boardman 1999; Green 2000; Darlington & Smulders 2001; García-Berthou 2001). To confound matters, there is no assurance that fresh weight is always a good indicator of fat reserves, or that fat reserves are a good indicator of fitness. For example, Rolff and Joop (2002) report that fresh weight in a dragonfly was a poor predictor of other fitness traits such as fat content, dry weight and muscle mass, and Ekman and Lilliendahl (1993) and Gosler (1996) found that fat storage was negatively correlated with social dominance in tits.

It is clear that size and energy reserves often play an important role in determining fitness. However the idea that a higher condition index equates to greater fitness is too simplistic. In some species, at some life history stages, in some environments, for some types of condition, these indexes may be strongly correlated with major components of fitness. Nonetheless, there is no reason to believe that this will always be the case. The ease of measuring condition indices has resulted in a plethora of studies reporting a positive correlation between "condition" and sexual ornament size (Andersson 1994; Johnstone 1995). But these relationships only provide weak evidence for condition dependence in the absence of experimental verification.

2.5 EXPERIMENTAL STUDIES OF CONDITION DEPENDENCE

Before reviewing the literature, I consider how to carry out a rigorous experimental study of condition dependence. There are three main areas of concern.

2.5.1 Comparison Of Traits

Statements concerning the condition dependence of sexual traits are of little value unless they refer to other traits against which ornaments can be compared; bluntly, "no controls, no conclusions" (Crawley 1993, p. 58). The handicap hypothesis assumes that sexual traits are subject to high differential costs unlike other traits, and that condition dependence is proportional to the cost differential (Iwasa *et al.*, 1991; Iwasa and Pomainkowski 1994, 1999). Sexual ornament expression should therefore show *heightened* condition dependence when compared with other traits.

This expectation is general, applying to any exaggerated sexual trait whether morphological or behavioural, and a contrast between the expression of sexual ornaments with that of non-sexual or control traits is the key feature that need to be addressed in tests of condition dependence.

Comparisons have been made using the homologous trait in females (if present), on the assumption that condition dependence of the female trait approximates that of the ancestral, unexaggerated state. Comparisons to male non-sexual traits are useful to make sure that differences in condition dependence are trait-specific (sexual *vs.* non-sexual) not sex-specific. Comparisons have also been made amongst sexual traits, when males have several ornamental traits or behaviours, to test whether those subject to stronger mate preference have higher condition dependence.

In all cases the prediction is that ornaments will have greater condition dependence than other traits, and this difference should increase as ornaments become more exaggerated. This requirement for a comparative approach to studying condition-dependence is not new. Zuk *et al.* (1990 p. 236) "measured several non-ornamental characters to test that ... [parasite-induced stress] ...should exert a *greater* effect on ornamental than non-ornamental traits, and that female choice should be focused specifically on male traits that best reveal information about male health" (emphasis mine). However, there are still far too many studies that simply report condition dependence just for a single, sexual trait.

2.5.2 Control Of Body Size

Many, if not all, traits show some degree of allometric scaling with body size. As a result, many, if not all, traits are likely to show some degree of condition-

dependent expression if body size covaries with condition. So removing the effects of body size variation is an important step in comparing the responses of sexual and non-sexual traits to changes in condition.

Sexual ornaments may just be exaggerated representations of body size, with body size being the main condition-dependent trait. In such circumstances we expect to see heightened sensitivity of ornaments to changes in body size, for instance by the evolution of elevated allometry (i.e. a higher value of b in the equation $Y = aX^b$, where Y = trait size and X = body size). Under these conditions, an incremental increase in body size leads to a proportionally greater increase in ornament size. Alternatively, ornaments may reflect a wider range of factors that influence condition, and then we expect part or all of their condition dependence to be independent of body size. In this scenario, ornaments are expected to show greater responses to experimental stress than other traits when the covariance with body size is removed. In general, the need to control for body size has been under appreciated in the condition dependence literature; assessing the importance of body size scaling will increase our knowledge of sexual signalling by revealing whether ornaments act as indices of body size, some other components of condition, or both.

In order to control for body size in the comparison of sexual and non-sexual traits one needs to know *a priori* how each trait scales with body size so that the covariance between each trait and body size can be appropriately removed. Controlling for body size has been attempted in a number of ways. A common method is to use relative trait size (individual trait size divided by body size) or trait size as a percentage of body size. However, these approaches are ineffective if scaling deviates from isometry, as the covariance with body size remains (Packard & Boardman 1999); in such cases any "condition dependence" can be purely artefactual.

Residuals derived from the regression of trait size on body size have also been used, but this procedure has been the subject of recent criticism (Kotiaho 1999; Green 2000; Darlington & Smulders 2001; García-Berthou 2001). This has led a number of authors to recommend including body size as a covariate in General Linear Models (Packard & Boardman 1999; Darlington & Smulders 2001; García-Berthou 2001). Such covariance analyses are free from the statistical drawbacks of other methods, and are effective at removing correlations with body size.

2.5.3 Experimental Manipulation Of Condition

Most experimental studies have used variation in environmental quality rather than in genetic quality to assess condition dependence of sexual ornaments. This is because easy and accurate control over environmental quality can usually easily be achieved under laboratory conditions. In contrast, it is hard to estimate genetic quality *a priori* or to set up distinct categories of genetic quality (but see Results). Typically two levels of environmental stress have been used, for example: food *vs.* no food or unparasitised *vs.* parasitized. Usually this allows low and high condition to be assigned unambiguously to the different stress levels.

In many cases, this approach has been sufficient to demonstrate a change in ornament size with condition. However, the use of only two groups is often problematic. First, the stress categories used may be unrelated to those experienced under natural conditions. In most cases, the range of stress experienced under natural conditions is not known with any degree of accuracy. But, truly unstressed animals are probably little more than laboratory artefacts, and many stressed groups are often exposed to environments at the extreme, or beyond, those to which they are adapted.

This means that the choice of stress treatments is crucial. If there is little difference between the two groups, there may be insufficient power to detect condition dependence, with the risk of declaring a false negative. If the two groups are exposed to extremely benign and extremely harsh stresses, then a response to treatment may be condition-dependent, but biologically unrealistic if the treatment range falls outside that found in nature. Such results would therefore be out of context with the trait's true signalling function, and one risks declaring a false positive with respect to the trait's evolved sensitivity.

One straightforward solution to these problems is to examine a much broader set of stress classes. Although this has the cost of increasing sample size, it permits more accurate assessment of the condition-dependent expression of sexual traits. The chances of detecting condition dependence are also enhanced, and it brings increased power for determining whether sexual traits exhibit heightened condition dependence. The timing of stress treatments poses similar problems. Stress can be applied continuously or at specific points during development. These different approaches tend to be appropriate for considering different questions. If continuous stresses are a frequent occurrence in nature then animals are likely to have adaptive response to them. So continuous stress experiments are likely to reveal the nature of adaptations to this type of stress. In contrast, brief stress shocks (e.g. an extreme temperature) are less predictable events, and may prove useful for determining which parts of development are sensitive to environmental stress.

2.6 METHODS

I surveyed published literature, restricting my compilation to experimental tests of sexual ornament condition dependence carried out using controlled variation of environmental or genetic factors. I used Johnstone (1995) to obtain references for literature published prior to 1995, and searched an electronic database (Web of Knowledge: http://wok.mimas.ac.uk) for relevant recent articles. I also consulted the reference lists of these papers to identify additional studies. Although not exhaustive, the review is large and represents the state of contemporary literature. In the survey I do not discuss correlations between ornaments and components of fitness as these relationships are beyond the scope of this chapter and have been subject of two recent reviews (Møller & Alatalo 1999; Jennions *et al.* 2001).

For each study I inferred an ordinal scale of condition using experimental groups and noted the type and number of treatments. I looked for comparisons between the response of sexual and non-sexual traits to treatment in order to test the hypothesis that the condition-dependence of ornaments is greater than that of other traits. Control traits were defined as such if they were similar in kind to ornamental traits and were of the same dimensions (where appropriate). For example, behavioural displays were not compared with non-sexual morphological traits. I also looked to see if traits had been compared after the influence of body size had been removed, and whether an effort had been made to investigate the genetic basis of condition dependence. Finally, my scale of condition was, in many cases, different to that of the original author(s) because I did not use indices of condition, and so some of my

conclusions differ from those reported in the source paper (e.g. Frischkneckt 1993; Birkhead *et al.* 1998).

2.7 RESULTS

2.7.1 General Findings

My review of the experimental literature comprised 55 studies covering 33 species in 25 genera from a wide range of vertebrate and invertebrate taxa (Table 2.1). 7 studies measured the effect of an environmental stress on two sexual characters, and 5 studies used 2 or more methods to investigate the condition dependence of sexual traits. A variety of different stresses were used, including food quantity and quality, parasite infection and brood manipulation. Of these studies (65 experiments in total), 49 report that sexual ornaments were condition-dependent; that is, the sexual trait showed decreased size in response to experimentally increased stress. 14 sexual ornaments were not condition-dependent under this definition.

Most studies (40/65) only employed two stress treatments, often limiting the analysis to a simple comparison of apparently "stressful" and "non-stressful" conditions. This procedure was sufficient to reveal sensitivity in the sexual trait in a number of species (Table 2.1). However, negative results are harder to interpret. It is noteworthy that most (12/14) of the studies that failed to find a condition-dependent response used only two treatments. To firmly establish that traits are not condition-dependent requires investigation of a wider range of stress levels and even different types of stress.

Only 20 studies compared the response of the sexual ornament with a suitable control trait. Of these, 15 studies confirmed that the sexual trait exhibited heightened sensitivity to stress in two species of cricket (Holzer *et al.* 2003; Scheuber *et al.* 2003a,b; Wagner & Hoback 1999), two stalk-eyed fly species (David *et al.* 1998, 2000; Knell *et al.* 1999), wolf spiders (Kotiaho 2000), guppy fish (Sheridan & Pomiankowski 1997; Grether 2000), two species of finch (Hill & Brawner 1998; Hill 2000; McGraw & Hill 2000), the brown-headed cowbird (McGraw *et al.* 2002) and red jungle fowl (Zuk *et al.* 1990). Sexual traits that failed to show heightened condition dependence include components of courtship in *Gryllus* crickets (Gray & Eckhardt 2001), male genitalic traits in water striders (Arnqvist & Thornhill 1998), and bill colour in zebra finches (Burley *et al.* 1992). In other studies, non-sexual trait expression was ignored.

About half (37/64) of the studies made appropriate adjustments for body size variation. This lack of control is worrying because correlated change of traits with body size could account for a large part of the condition-dependent response observed. This adjustment is unlikely to be important for some traits like colour and behaviour which are not expected to covary with body size. This is often assumed; it would be better if it were demonstrated (e.g. Scheuber *et al.* 2003a; Kotiaho 2002).

Surprisingly, studies using genetic information are rare (Table 2.1), despite the importance of genes in the proposed function of sexual ornaments. Several studies used a split brood design, in which brothers were either stressed or unstressed (7 studies), simply as a control for genetic effects. Two studies used inbreeding as a form of genetic stress (Sheridan & Pomiankowski 1997; Oosterhout *et al.* 2003). Of greater interest, a few studies used pedigree information to investigate elements of the handicap hypothesis. In dung beetles, manipulation of environmental quality revealed

that courtship is condition-dependent as adult males kept on excess dung had higher courtship display rates than males experimentally deprived of dung (Kotiaho *et al.* 2001; Kotiaho 2002). Kotiaho *et al.* (2001) also used a half-sib design to show that display rate was genetically correlated to offspring residual mass (a measure of condition). Unfortunately, no comparisons with non-sexual behaviour were made. A different point was investigated by comparing half- and full-sib stalk-eyed fly families under a range of food stresses (David *et al.* 2000). This revealed genetic variation underlying condition-dependent expression of the male sexual ornament; some families produced large male eyespan under all conditions, whereas others showed a consistent decline in male eyespan as stress increased. However, an experiment using waxmoths by Jia *et al.* (2000) found that different genotypes (lines artificially selected for high or low values of two sexual traits) predominantly showed crossing-over of reaction-norms between control and non-standard environments (i.e. line ranks tended to be reversed in the non-standard treatments). This suggests that genetic specialization and trade-offs dominate sexual trait expression rather than condition-dependent quality variation. However, these findings are difficult to interpret from the perspective of condition dependence, as it is unclear whether the non-standard environments were more or less stressful than the standard rearing procedure.

To clarify the current state of experimental work, I discuss in greater detail the merits and shortcomings of research in the 7 taxa in which there is good evidence for heightened condition dependence of sexual ornaments. A commentary is also provided on some interesting findings in dung beetles, although evidence for heightened condition dependence is less well supported in this taxon. These experiments are not without problems, so an objective is to show where more work is

needed. For instance, few studies have taken into account body size covariation, examined more than two levels or different types of stress, and investigated the genetic basis of condition dependence.

2.7.2 Crickets

Male crickets produce three types of acoustic sexual signal: a long-range calling song to attract females at a distance, a courtship song to persuade attracted females to mate, and an aggressive song used during encounters with neighbouring males (Alexander 1961). Wagner and Hoback (1999) investigated the condition dependence of male calling song in *Gryllus lineaticeps* by maintaining adults on high or low quality food. They measured five song parameters and found that males called more frequently and had higher chirp rates when reared on a high quality diet compared to brothers reared on poorer diets. Chirp and pulse duration, and dominant frequency were unaffected by treatment. Similarly, Scheuber *et al.* (2003a) found that the frequency of calling and chirp rate both declined as adult dietary stress increased in the congener *G. campestris*. Again, other song characteristics (chirp duration, syllable number, chirp intensity and carrier frequency) were unaffected by adult diet.

The biological relevance of these laboratory-based findings about calling song was confirmed in an experimental field study. Holzer *et al.* (2003) increased male condition in wild *G. campestris* by supplementing food in a confined area close to the burrow. Males with an augmented food supply called more frequently than a group of control males. No effect of treatment was found on any other song characteristic. In addition, food supplemented males attracted more females than did control males, and this was at least partially attributable to their elevated calling rate.

Stress during nymphal developmental has also been experimentally investigated in *G. campestris* (Scheuber *et al.* 2003b). Individuals raised on a poor nymphal food source produced calling song with a higher carrier frequency when adults. This was caused by a disproportionate reduction in the stridulatory harp area with respect to body size. These males were less attractive, as females prefer to mate with males that produce low frequency calls. No other song characteristic responded to nymphal stress. It is interesting to note that carrier frequency was not affected by adult stress, as this is fixed via harp size at the final moult (Scheuber *et al.* 2003b). The reverse pattern is seen for chirp rate, which showed no response to stress during the nymphal stage but was strongly affected by adult dietary manipulation (Scheuber *et al.* 2003a).

In contrast to these reports of condition dependence in calling song, there has been a failure to show condition dependence in courtship song. Gray and Eckhardt (2001) reared *G. texensis* nymphs and adults on high or low quality diets. No effect of diet was found at either life-history stage for any of the three courtship song characteristics measured (interphase interval, chirp rate and the number of low frequency chirps). Since courtship song was also unrelated to two estimates of condition (residual mass and fat reserves), Gray and Eckhardt (2001) concluded that courtship song was not condition-dependent. A similar lack of condition dependence was found for courtship song in *G. lineaticeps*, although only one aspect of courtship song was measured (chirp rate) and individuals were only stressed in the adult phase (Wagner & Reiser 2000).

Crickets acoustic signals are complex multi-component traits. Calling song was condition-dependent, but courtship song was not. Only certain elements of the calling song showed condition dependence (e.g. frequency of calling, chirp rate and

carrier frequency), whilst others did not (e.g. chirp duration, syllable number, chirp intensity). In addition, the timing of stressful conditions (nymphal *vs.* adult) caused different song elements to respond. Taken together, these experimental results suggest that some elements of the call show heightened condition dependence. These heightened responses are contingent on the time at which the traits are expressed. Other call characteristics do not appear to be condition-dependent and probably have different signalling functions (e.g. species recognition), although it remains possible that they signal other types of condition that were not tested (e.g. parasite resistance). The strength of these experiments lies in the use of multiple elements of the acoustic signal to identify those that are strongly condition-dependent against non-responding "controls".

2.7.3 Stalk-Eyed Flies

Stalk-eyed flies (Diopsidae: Diptera) show elongation of the head capsule into long processes ('eye-stalks') onto which the eyes and antennae are laterally displaced. Both sexes possess some degree of eye-stalk elongation (Wilkinson & Dodson 1997; Baker *et al.* 2001), but in many species male eyespan is much greater than that of females. Some species have no sex differences however, and eyespan monomorphism is believed to be plesiomorphic, with sexual dimorphism having evolved independently many times (Baker & Wilkinson 2001).

The main stalk-eyed fly model is *Cyrtodiopsis dalmanni*, a highly sexually dimorphic species inhabiting the forests of South-East Asia. Nocturnal mating aggregations form on root hairs overhanging the banks of streams (Burkhardt & de la Motte 1985; Wilkinson & Dodson 1997), and males fight for control of these roosting

sites. Contests are usually won by males with the largest eyespan (Burkhardt & de la Motte 1983, 1987; Wilkinson & Dodson 1997; Panhuis & Wilkinson 1999), and females prefer to roost and mate with males possessing the largest absolute and largest relative eyespan (Wilkinson & Reillo 1994; Hingle *et al.* 2001). Male eyespan in *C. dalmanni* is therefore subject to strong inter- and intra-sexual selection.

David *et al.* (1998) investigated the condition dependence of male eyespan by rearing larvae from two separate populations on one of five food stress levels by varying the amount of food available to a given number of eggs. Increasing larval density caused male eyespan to decline in both populations. Larval stress had similar effects on female eyespan as well as male and female wing size. Importantly, David *et al.* (1998) demonstrated that the ornament exhibited heightened condition dependence, as male eyespan was significantly more sensitive to changes in larval density than the homologous female trait. Such patterns remained when relative trait size (the ratio of eyespan-to-body size) was investigated and when body size was controlled as a covariate in a General Linear Model. However, David *et al.* (1998) used wing size as both a non-sexual trait and a body size index. It is therefore uncertain whether condition dependence arose through changes in the relationship of wing size-to-body size or eyespan-to-body size. A similar result was found across two larval food quality stress regimes in another dimorphic Diopsid, *Diasemopsis aethiopica* (Knell *et al.* 1999). By varying food quality (high *vs.* low), Knell *et al.* (1999) showed that males in high condition produced larger eyespans for their body size than males in low condition. In contrast, females in high condition invested more in both eyespan and body size suggesting that at least some of the change in female eyespan was the result of correlated change in body size; diet had no effect on male

body size. Thus male eyespan appears to be more sensitive to condition than female eyespan in *D. aethiopica.*

A further study in *C. dalmanni* showed that there was genetic variation underlying the response of male eyespan to food stress (David *et al.* 2000). This experiment used a full- and half-sib design, exposing larvae to 3 food quality types. Some genotypes maintained large male eyespan under all environments, whilst others became progressively smaller as stress increased. This pattern persisted when body size variation was controlled for using relative trait values. However, female eyespan, and wing length in both sexes showed no or little genetic condition-dependent response once body size had been controlled for. It was also noted that the sexual trait amplified differences between genotypes under stressful environmental conditions, even though the mean trait size declined (David *et al.* 2000). Again this pattern persisted using relative trait values. Non-sexual control traits, female eyespan and wing length in both sexes, did not show any change in genetic variation across environments.

David *et al.* (2000) overcame their earlier (1998) problem of scaling uncertainty by using thorax length as a measure of body size and looking at the responses of both eyespan and wing traits relative to thorax length. Unfortunately, their use of relative trait size (i.e. dividing by thorax length) did not fully remove the covariance with body size, because traits do not scale isometrically with thorax and exhibit non-zero intercepts (the intercept of male eyespan is more negative than that other traits).

2.7.4 Wolf Spiders

Male wolf spiders (*Hygrolycosa rubrofasciata*) court females by drumming dry leaves with their abdomen, and females mate preferentially with males drumming at the highest rate (Parri *et al.* 1997). Kotiaho (2000) investigated the condition dependence of drumming rate by keeping males on three food (quantity) regimes in both the presence and absence of females. The male sexual trait was therefore assayed under conditions when it had little sexual signalling value (females absent) and when it had high sexual signalling value (females present). All males increased their drumming rate when females were present. However, there was a disproportionate increase in drumming amongst males reared on better food. Thus drumming rate in male *H. rubrofasciata* shows heightened condition dependence; when used, its signalling function is elevated. Note that this experimental design cleverly allowed the sexual trait to act as its own control in the absence of females.

2.7.5 Guppy

Sheridan and Pomiankowski (1997) used inbreeding to generate genetic stress in two populations (Paria and Aripo) of male Trinidadian guppies (*Poecilia reticulata*). Males from the Paria population have bright carotenoid coloration and Paria females show strong preference for this trait (Houde 1988a; Houde & Endler 1990). Aripo females do not prefer males with high carotenoid colouration, and Aripo males are less ornamented (with respect to carotenoid colouration) than Paria males (Houde 1988b).

Replicate inbred and control lines were created for each population (Sheridan & Pomiankowski 1997). Inbreeding had little effect on morphological characters from either population. The sexual trait in Paria males, carotenoid colouration, was significantly reduced in inbred relative to control lines and this difference persisted after controlling for body size. There was no difference between inbred and control lines in two coloration traits that are thought to be of lesser importance in mate choice: melanin pigmentation and the number of pigment spots (Houde 1988a; Houde & Endler 1990). The sexual trait was thus more sensitive to moderate genetic stress than non-sexual traits. In contrast, inbred Aripo males did not differ from controls in the amount of carotenoid coloration, but inbred males had reduced melanin and spot numbers. Whether these latter two traits serve as sexual attractants in the Aripo population is not known (Houde 1988b).

Similar results have recently been reported for another population of guppies from the Tacarigua river in Trinidad (Oosterhout *et al.* 2003). The area of male carotenoid and melanin coloration, and the frequency of male courtship sigmoid displays showed strong inbreeding depression after two generations of inbreeding. Unfortunately no outbred controls were bred to test for the effect of laboratory rearing. In addition, no comparisons were made between traits, so it is not possible to conclude that there is heightened sexual trait condition dependence.

In another study on Trinidadian guppies, Grether (2000) manipulated dietary carotenoid levels and examined the response of male pigmentation in a number of different populations. After controlling for body size, several measures of male carotenoid coloration responded to diet, whereas other pigmentation did not. As carotenoid coloration is important in sexual attraction, this study shows that the sexual traits were more sensitive to dietary treatment than other pigments.

Importantly, males from different populations reacted more or less strongly to the dietary treatment, suggesting genetic variation in the response. However, the diets only differed in carotenoid content. So while this study demonstrates heightened carotenoid-dependent expression in carotenoid based coloration, it remains unclear how this relates to condition. Similar carotenoid-limited findings also have been reported in another guppy experiment (Kodric-Brown 1989) and in finches (Hill 1992, 1993; Blount *et al.* 2003a).

2.7.6 Finches And Cowbirds

Hill (2000) found that food stress modulated the ability of male house finches (*Carpodacus mexicanus*) to process different carotenoids from the diet into plumage colouration. Males were collected males as juveniles from the wild and subjected them to high or low food, with abundant or restricted carotenoid content (2 × 2 design). In general, males on poor diets were less able to convert dietary carotenoids into colourful ornaments. In contrast, diet had no discernable effect on the deposition of melanin pigmentation in tail feathers. Likewise, nutritionally deprived male brown-headed cowbirds (*Molothrus ater*) grew significantly less green, less saturated and less bright iridescent plumage than did birds fed *ad libitum*, but this treatment had no effect on melanin pigmentation (McGraw *et al.* 2002).

In another study using house finches, Hill and Brawner (1998) investigated the effect of parasitism on carotenoid coloration. They found that juvenile males experimentally infected with a modest level of coccidians (*Isospora* species and/or *Mycoplasma gallicepticum*) developed plumage that was less red and had less saturated carotenoid coloration than juveniles treated to have sub-clinical infections.

Again, there was no effect of infection status on melanin pigmentation in tail feathers. Similar experimental findings have been reported for the expression of carotenoid and melanin pigmentation in parasitized and unparasitised male American goldfinches (*Carduelis tristis*, McGraw & Hill 2000). Together these studies provide good evidence for heightened condition dependence of carotenoid/iridescent pigmentation compared to melanin pigmentation.

2.7.7 Red Jungle Fowl

Male jungle fowl (*Gallus gallus*) possess a number of secondary sexual traits that are attractive to females, such as elongated and ornamental feathers and coloured facial features (comb and iris). Zuk *et al.* (1990) experimentally divided chicks into two groups and administered a dose of the intestinal roundworm *Ascaridia galli* to one group (at a naturally occurring magnitude). At maturity, a suite of sexual and non-sexual male traits were measured and canonical discriminant analysis was applied to each set of traits to see if group membership (i.e. parasitized or control) could be predicted using several characters simultaneously. Control males, in general, had more exaggerated and elaborate ornamental plumage and brighter sexual coloration than parasitised males. However, the same analysis performed on a set of non-sexual traits (tarsus length, bill length, bill width, length of saddle feathers) showed no significant separation for parasitised and control roosters. The presence of roundworm therefore had a disproportionate effect on ornamental traits.

2.7.8 Dung Beetles

Male dung beetles (*Onthophagus* sp.) have two types of sexually selected trait, courtship display and horns. Male courtship display increases the probability of mating (Kotiaho *et al.* 2001) and this effect is independent of horn morphology or body size in the absence of male conspecifics (Kotiaho 2002). Manipulation of environmental quality revealed that courtship is condition-dependent as adult males kept on excess dung had higher courtship display rates than males experimentally deprived of dung (Kotiaho *et al.* 2001; Kotiaho 2002). Unfortunately, no comparison with non-sexual behaviour was made. Courtship rate is heritable, and Kotiaho *et al.* (2001) used a half-sib design to show that display rate was genetically correlated to offspring residual mass (a measure of condition) in *O. taurus*. The importance of residual mass in *O. taurus* is unknown, but it exhibits high levels of additive genetic variance either when measured as standardised residual mass (Kotiaho *et al.* 2001) or, more appropriately, as somatic weight using body size as a covariate (Simmons & Kotiaho 2002), suggesting that residual mass may make a major contribution to fitness (Houle 1992; Pomiankowski & Møller 1995).

Male Onthophagines also exhibit dimorphic horn morphology. Males larger than a critical body size develop disproportionately long horns on their heads, whilst smaller males develop rudimentary horns or none at all (Emlen 1994; Hunt & Simmons 1997; Moczek & Emlen 1999). Manipulation of condition by alteration of food quantity showed that horn expression is condition-dependent (Emlen 1994; Hunt & Simmons 1997). Beetles reared as larvae on small amounts of dung had small body size and did not usually develop horns, whereas those given large amounts of dung

had large body size with long horns. Although there was no explicit comparison of horn expression with that of non-sexual traits, it is probable that horns have heightened condition dependence because of their unusual (sigmoidal) allometry.

Horn length was deemed unlikely to signal genetic benefits as neither body size or horn length was found to be heritable in laboratory studies; variation in each trait was entirely attributed to larval dung quantity (Emlen 1994; Moczek & Emlen 1999). However, recent research by Kotiaho *et al.* (2003) has suggested that the heritability of male offspring morphology is strongly affected by a sire-mediated maternal component. Female *O. taurus* differentially provision their progeny depending on the phenotype of their mate; mothers provide more resources to offspring when mated with large-horned males. Kotiaho *et al.* (2003) speculated that these differential "maternal effects" were in fact an indirect sire effect mediated by the transfer of fitness-enhancing seminal products to females during mating, as, contrary to life-history expectations, females had an elevated lifespan and increased reproductive investment when mated to large-horned males.

The absence of female preference for horns (Kotiaho 2002) suggests that horns are inter-specific weapons rather than sexual ornaments (Moczek & Emlen 2000; Kotiaho 2002). Horned males aggressively defend tunnels containing a breeding female and contests are usually won by the male with the biggest horns (Moczek & Emlen 2000). In contrast, hornless males are more agile and adopt a sneaking strategy to gain copulations (Emlen 1997; Moczek & Emlen 2000). So variation in male size and horn morphology results in part from frequency-dependent selection on different male strategies and is probably not an example of a condition-dependent handicap.

2.8 DISCUSSION & FUTURE DIRECTIONS

Despite the common claim that ornaments are condition-dependent, the unexpected conclusion from my literature review is that there is little support from well-designed experiments. Based on the minimum criterion that sexual traits were shown to be more sensitive to stress than non-sexual traits, only experiments in 10 species support this claim: two cricket species, two stalk-eyed fly species, wolf spiders, guppies, two species of finch, brown-headed cowbirds and red jungle fowl (Zuk *et al.* 1990; Sheridan & Pomiankowski 1997; David *et al.* 1998, 2000; Hill & Brawner 1998; Knell *et al.* 1999; Wagner & Hoback 1999; Hill 2000; Kotiaho 2000; McGraw & Hill 2000; McGraw *et al.* 2002; Holzer et al. 2003; Scheuber *et al.* 2003a,b). I do not conclude that condition dependence is rare; rather that good experimental support is lacking in the majority of species studied. More rigorous studies are needed to fully test the prediction of *heightened* condition dependence in sexual ornaments made by the handicap hypothesis.

There are a number of key elements that need to be addressed in an experimental study. First, the response of the male sexual trait needs to be assessed relative to traits under weaker or no sexual selection. The latter could be the homologous trait in females, or other traits in the male that do not have signalling functions. A possible additional comparison is within or between ornaments, as different elements of courtship display may have evolved to signal different characteristics (Møller & Pomiankowski 1993; Pomiankowski & Iwasa 1993; Iwasa & Pomiankowski 1994). Temporal comparisons of sexual trait condition dependence can also be made when the value of the signal differs over time (e.g. Kotiaho 2000). Second, proper account of body size scaling needs to be undertaken as most traits

scale with body size. This is important not just for the proper comparison of sexual ornaments and non-sexual traits. It is also informative in establishing whether sexual ornaments signal something other than body size. Third, a suitable number of stresses need to be examined (3-5 is usually sufficient), within the normal range experienced under natural conditions. It is not enough to compare extreme stress and no stress. It is also useful to investigate several types of environmental stress or stress applied at different times during development which are likely to relevant to the species under study. Unfortunately, few studies satisfy all these conditions.

How is this field of investigation likely to develop over the next decade? One area that needs far more development is the study of the genetic basis of condition-dependent expression. The paucity of genetic experiments greatly limits how the phenotypic data can be interpreted. Whilst the handicap principle can still work in the absence of a genetic advantage (Price *et al.* 1993; Iwasa & Pomiankowski 1999), much of the controversy in sexual selection is concerned with whether male ornaments signal inherited viability benefits. If exaggerated sexual ornaments signal male genetic quality, we expect a genetic basis to condition dependence. In particular, we expect males with higher quality genotypes to have larger sexual ornaments. This can be tested directly, where there is independent evidence that different genotypes vary in fitness or in major components of fitness. For example, experimental manipulation of mutation load would be expected to induce greater changes in sexual traits relative to non-sexual traits when compared with a control group reared under the same environment. Indirect tests are also possible by searching for genetic variation in condition dependence. In addition, the interaction between environmental and genetic variation is important (Griffith *et al.* 1999). Condition, like other life history traits, is expected to exhibit a large component of environmental variance

(Price & Schluter 1991; Houle 1992). As a result of heightened condition-dependent expression, ornaments are also expected to possess large components of environmental variance. This means that genotype by environment interactions are crucial for our understanding of sexual traits, as they determine to what degree ornaments signal heritable benefits. The handicap hypothesis predicts that males with high genetic quality should do well in all environments, and their advantage in terms of ornament size should be amplified under harsh environmental conditions, as these increase the differential costs of signalling. Alternatively, the genetic variation underlying sexual ornament size may reflect local adaptation to particular environmental conditions. So far, one experimental study supports each of these possibilities (David *et al.* 2000; Jia *et al.* 2000), but more are required to examine their generality.

Related to these points is the need for an understanding of the actual genes and other proximate mechanisms involved in determining condition dependence. One would like to know which aspects of condition are being signalled, and whether the underlying mechanisms involved in signalling are organism specific or general across taxa. It will also be important to understand how the gene networks underlying ornament expression evolve for it to signal condition

In this review, I have emphasized the need for experimental studies. But conversely these need to relate back to behaviour in the field. It is often unclear whether the environmental stresses investigated are relevant to the natural ecology of the species involved. The best evidence on this should come from long-term field studies, like that of the collared flycatcher, where brood manipulation has been used to measure how environmental stress affects the expression of the white forehead patch (Gustafsson *et al.* 1995; Qvarnström 1999; see also Griffith 2000).

Another area that seems ripe for investigation is the evolutionary pattern of condition dependence. In groups with large numbers of species, it should be possible to test for ecological and reproductive correlates of increased condition dependence in sexual traits. For example, the extensive changes in stalk-eyed fly eyespan exaggeration or in guppy fish coloration and concomitant changes in female mate preferences (Houde & Endler 1990; Wilkinson & Dodson 1997; Baker & Wilkinson 2001), are predicted to be linked to alterations in sexual trait condition dependence. Accordingly, phylogenetic tests should be designed to detect an evolved change in condition dependence rather than its existence *per se*. This kind of comparative analysis has not yet been attempted.

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Table 2.1. Experimental investigations into the condition dependence of sexual traits.

Species		Sexual Trait	Control Trait	Sexual Trait CD[1]	Control Trait CD	Sexual Trait CD > Control Trait CD	Controlled for Body Size	Stress (*n*)	Genetic Design	Genetic Effect	Reference
Insects, Arachnids and Crustaceans											
Cricket	*Gryllus campestris*	calling song	song components	✓	×	✓[2]	✓[3]	A (2)	×	-	Holzer *et al.* 2003
	G. campestris	calling song	song components	✓	×	✓[2]	✓[4]	A (3)	✓ (SB)	-	Scheuber *et al.* 2003a
	G. campestris	calling song/harp size	song components	✓	×	✓[2]	✓[5]	B (2)	✓ (FS)	✓ (G)	Scheuber *et al.* 2003b
	G. lineaticeps	calling song	song components	✓	×	✓[2]	×	B (2)	✓ (SB)	-	Wagner & Hoback 1999
	G. lineaticeps	courtship song	×	×	-	-	×	B (2)	✓ (SB)	-	Wagner & Reiser 2000
	G. texensis	courtship song	song components	×	×	×	×	B (2)	×	-	Gray & Eckhardt 2001
Damselfly	*Mnais costalis*	wing pigmentation	×	✓	-	-	×	A (2)	×	-	Hooper *et al.* 1999
Dung beetle	*Onthophagus taurus*	courtship rate	×	✓	-	-	✓[3]	A (2)	✓ (HS)	✓ (G)	Kotiaho *et al.* 2001
	O. taurus	horn length	×	✓	-	?[6]	✓[7]	A (4)	×	-	Hunt & Simmons 1997
	O. acuminatus	horn length	×	✓	-	?[6]	✓[7]	A (2)	✓ (PO)	× (G)	Emlen 1994
	O. taurus, O. binodis, O. australis	courtship rate	×	✓	-	-	✓[4,5]	A (2)	×	-	Kotiaho 2002
Fruit fly	*Drosophila grimshawi*	courtship display	×	✓	-	-	×	B (2)	×	-	Droney 1996
Grain beetle	*Tenebrio molitor*	pheromone	×	✓	-	-	×	A (2)	×	-	Rantala *et al.* 2003
Stalk-eyed fly	*Cyrtodiopsis dalmanni*	eyespan	female eyespan	✓	×	✓	✓[5]	A,C (5)	×	-	David *et al.* 1998
	C. dalmanni	eyespan	wing, female eyespan	✓	×	✓	✓[8]	B (3)	✓ (HS,FS)	✓ (G×E)	David *et al.* 2000
	Diasemopsis aethiopica	eyespan	female eyespan	✓	✓	✓	✓[5]	B (2)	×	-	Knell *et al.* 1999
Water strider	*Gerris incognitus*	genitalia	morphological traits	✓	✓	×	×	A,B (2)	✓ (FS,BIP)	✓ (G)	Arnqvist & Thornhill 1998
Waxmoth	*Achroia grisella*	acoustic signal rate	×	?[9]	-	-	×	A,B,C,D,E (6)	✓ (AS)	✓ (G×E)	Jia *et al.* 2000
		acoustic peak amplitude	×	?[9]	-	-	×	A,B,C,D (4)	✓ (AS)	✓ (G)	
Wolf spider	*Hygrolycosa rubrofasciata*	drumming rate	×	✓	-	-	✓[3]	A (3)	×	-	Mappes *et al.* 1996
	H. rubrofasciata	drumming rate (female present)	drumming rate (female absent)	✓	✓	✓	✓[3]	A (3)	×	-	Kotiaho 2000
Fiddler crab	*Uca beebei*	mud pillar building	×	✓	-	-	×	A (2)	×	-	Backwell *et al.* 1995
	U. annulipes	claw waving	×	✓	-	-	×	A (3)	×	-	Jennions & Backwell 1998
	U. lactea	semidome building	×	✓	-	-	✓[4]	A (3)	×	-	Kim & Choe 2003
		claw waving	×	✓	-	-	✓[4]	A (3)	×	-	
Fish and Amphibians											
Guppy	*Poecilia reticulata*	carotenoid pigmentation	×	✓	-	-	✓[10]	F (2)	✓ (SB)	✓ (G)	Kodric-Brown 1989
	P. reticulata	carotenoid pigmentation	×	✓	-	-	✓[5]	G (2)	✓ (SB)	-	Houde & Torio 1992
	P. reticulata	carotenoid pigmentation	morphological traits, melanin pigmentation	✓	×	✓	✓[11]	H (2)	✓ (IB)	✓ (G)	Sheridan & Pomiankowski 1997
	P. reticulata	carotenoid pigmentation	morphological traits, other pigmentation	✓	×	✓	✓[8]	F (3)	✓ (IPV,FS)	✓ (G×E)	Grether 2000
	P. reticulata	carotenoid pigmentation	melanin pigmentation	✓	✓	-	✓[11]	H (3)	✓ (IB)	-	Van Oosterhout *et al.* 2003
		courtship display	×	✓	-	-	×	H (3)	✓ (IB)	-	
Three-spined stickleback	*Gasterosteus aculeatus*	carotenoid pigmentation	×	✓	-	-	×	G (2)	×	-	Milinski & Bakker 1990
	G. aculeatus	carotenoid pigmentation	×	×	-	-	✓[11]	A (2)	×	-	Candolin 1999
	G. aculeatus	carotenoid pigmentation	×	×	-	-	✓[11]	A (2)	×	-	Candolin 2000
	G. aculeatus	carotenoid pigmentation	blue eye pigmentation	×	-	-	✓[5]	A (5)	×	-	Frischknecht 1993
Smooth newt	*Triturus vulgaris*	dorsal crest	×	✓	-	-	×	A (2)	×	-	Green 1991
Birds											
American goldfinch	*Carduelis tristis*	carotenoid pigmentation	melanin pigmentation	✓	×	✓	✓[12]	G (2)	×	-	McGraw & Hill 2000
Barn swallow	*Hirundo rustica*	tail length	×	✓	-	-	✓[3]	G (3)	×	-	Møller 1991
Brown-headed cowbird	*Molothrus ater*	iridescent plumage	melanin pigmentation	✓	×	✓	✓[10]	A (2)	×	-	McGraw *et al.* 2002
Collared flycatcher	*Ficedula albicollis*	forehead patch	×	✓	-	-	×	J (5)	×	-	Gustafsson *et al.* 1995
			×	✓	-	-	✓[3]	K (5)	×	-	
	F. albicollis	forehead patch	×	×	-	-	×	J (3)	✓ (F/MS)	✓ (G×E)	Qvarnström 1999
European starling	*Sturnus vulgaris*	song	×	✓	-	-	×	L (2)	×	-	Buchanan *et al.* 2003
House finch	*Carpodacus mexicanus*	carotenoid pigmentation	×	✓	×	-	×	F (3)	×	-	Hill 1990
	C. mexicanus	carotenoid pigmentation	×	✓	×	-	×	F (2/3)	×	-	Hill 1992
	C. mexicanus	carotenoid pigmentation	×	✓	-	-	×	F (2/3)	✓ (IPV)	✓ (G)	Hill 1993
	C. mexicanus	carotenoid pigmentation	melanin pigmentation	✓	×	✓	×	G (2)	×	-	Hill & Brawner 1998
	C. mexicanus	carotenoid pigmentation	×	✓	-	-	✓[10]	G (4)	×	-	Brawner *et al.* 2000
	C. mexicanus	carotenoid pigmentation	melanin pigmentation	✓	×	✓	×	A+F (5)	×	-	Hill 2000
House sparrow	*Passer domesticus*	melanin pigmentation	×	×	-	-	×	B (2)	×	-	Gonzalez *et al.* 1999
	P. domesticus	melanin pigmentation	×	✓	-	-	✓[3]	J (5)	×	-	Griffith 2000
	P. domesticus	melanin pigmentation	×	×	-	-	✓[10]	A (2)	×	-	McGraw *et al.* 2002
Mallard	*Anas platyrhynchos*	display activity	×	✓	-	-	✓[10]	A (2)	×	-	Holmberg *et al.* 1989
		plumage status	×	×	-	-	✓[10]	A (2)	×	-	
Red jungle fowl	*Gallus gallus*	plumage & comb characteristics	tarsus length, bill size, saddle feathers	✓	×	✓	×	G (2)	×	-	Zuk *et al.* 1990
Ring-necked pheasant	*Phasianus colchicus*	wattle	×	✓	-	-	✓[5]	B (2)[13]	×	-	Ohlsson *et al.* 2001
		spurs	×	×	-	-	✓[5]	B (2)[13]	×	-	
Zebra finch	*Taeniopygia guttata*	bill colour	female bill colour	✓	✓	×	✓[3]	B (2)[14]	×	-	Burley *et al.* 1992
			×	×	-	-	✓[3]	B (2)	×	-	
			×	✓	-	-	×	C (2)	×	-	
	T. guttata	bill colour	×	✓	-	-	✓[5]	F (2)	×	-	Blount *et al.* 2003a
	T. guttata	bill colour	×	×	-	-	✓[10]	B (2)	×	-	Blount *et al.* 2003b
	T. guttata	bill colour	×	×	-	-	×	B+I (2)	✓ (SB)	-	Birkhead *et al.* 1998
		song rate	×	×	-	-	×	B+I (2)	✓ (SB)	-	
Mammals											
Red deer	*Cervus elaphus*	antlers	×	✓	-	-	×	A (2)	×	-	Suttie 1980 (cited in Andersson 1994)

Notes:

[1] – CD = condition dependence
[2] – Some parameters of calling song show condition dependence with specific stresses whilst others do not. This suggests that different components of the sexual trait show heightened condition dependence under some circumstances.
[3] – repeated measures
[4] – no correlation with body size
[5] – body size included as a covariate
[6] – Horn length shows sigmoidal allometry unlike other traits, which is interpreted as elevated condition dependence, but horns are thought to be weapons rather than sexual ornaments (Kotiaho 2002), and under frequency-dependent rather than sexual selection (Moczek & Emlen 2000).
[7] – via allometry (see [6])
[8] – trait size divided by body size
[9] – evidence unclear (see text)
[10] – no size difference between treatments
[11] – % coverage
[12] – residuals from regression of ornament on body size
[13] – 2 treatments applied either in early or late growth periods
[14] – wild vs. supplemented aviary birds

Stress:

n – number of stresses
A – diet (quantity)
B – diet (quality)
C – density
D – temperature
E – photoperiod
F – carotenoid availability (diet otherwise constant across treatments)
G – parasites
H – inbreeding (genetic stress)
I – exercise
J – brood size manipulations
K – reproductive effort
L – predictability of food supply

Genetic Design:

SB – split brood
HS – half-sib
PO – parent/offspring
FS – full-sib
AS – artificial selection
BIP – bi-parental progenies
F/MS – father on mid-son
IPV – inter-population variation when reared under standard conditions
IB – inbreeding

Genetic Effects:

G – genotype effect
G×E – genotype × environment interaction

3

Condition Dependence Of Sexual Ornament Size And Variation In The Stalk-Eyed Fly *Cyrtodiopsis dalmanni* (Diptera: Diopsidae)

3.1 ABSTRACT:

I used the stalk-eyed fly *Cyrtodiopsis dalmanni* to examine predictions made by condition-dependent handicap models of sexual selection. Condition was experimentally varied by manipulation of larval food availability. *C. dalmanni* is a highly dimorphic species exhibiting strong sexual selection, and the male sexual ornament (exaggerated eyespan) showed strong condition-dependent expression relative to the homologous trait in females and non-sexual traits. Male eyespan also showed a great increase in standardized variance under stress, unlike non-sexual traits. The inflated variance of the male ornament was primarily attributable to condition-dependent (but body size independent) increase in variance. Thus evaluation of male eyespan allows females to gain additional information about male condition over and above that given by body size. These findings accord well with condition-dependent handicap models of sexual selection.

3.2 INTRODUCTION

The handicap hypothesis of sexual selection proposes that sexual ornament size reveals male condition, thereby allowing females to choose mates with higher phenotypic or genotypic quality (Zahavi 1975; Andersson 1986; Pomiankowski 1987, 1988; Grafen 1990; Iwasa *et al.* 1991; Iwasa & Pomiankowski 1994, 1999). Condition-dependent costs ensure that male ornaments are honest signals of quality (i.e. costs are higher for lower quality individuals). This leads to the prediction that ornaments should have condition-dependent expression.

Although there is general support for this, much of the evidence is correlational (reviewed in Andersson 1994; Johnstone 1995; Cotton *et al.* 2004; Chapter 2) and these associations are confounded by many uncontrolled factors. There are relatively few studies using experimental manipulations of condition under controlled conditions and most of these suffer from three common design problems that constrain their interpretation. First, most experimental studies have only assayed sexual trait expression (e.g. Kodric-Brown 1989; Green 1991; Houde & Torio 1992; Ohlsson *et al.* 2002). The handicap hypothesis assumes that sexual traits are subject to high differential costs unlike other traits. Sexual ornament expression should therefore show *heightened* condition dependence when compared with other traits (Iwasa & Pomiankowski 1994, 1999). This is the key feature that needs to be addressed in tests of condition dependence and is general, applying to any exaggerated sexual trait whether morphological or behavioural (e.g. Kotiaho 2000). Second, since body size tends to covary positively with condition and traits generally scale with body size in many species (Stern & Emlen 1999), most traits will automatically decline with increasing stress. So it is important that appropriate control

for body size is undertaken when comparing sexual and non-sexual traits. Controlling for body size also reveals whether sexual ornaments are just exaggerated representations of body size, or whether their expression reflects a wider range of condition factors that act independently of body size. Finally, sexual traits are often assayed only over two arbitrarily defined levels of condition (e.g. Kodric-Brown 1989; Milinski & Bakker 1990; Green 1991; Houde & Torio 1992; Candolin 1999; Hooper *et al.* 1999; Ohlsson *et al.* 2002), for example parasitized *vs.* not parasitized, low food *vs.* high food. Often these levels are at the extremes, or even beyond, the normal range encountered under natural conditions. Two levels are typically insufficient to test whether sexual traits show heightened levels of condition-dependent expression as they only allow a single comparison between condition types and it is often not always clear that condition has been successfully manipulated. It is important to investigate the response of traits over a broad range of conditions that reflect those experienced under natural conditions.

Another prediction of the handicap hypothesis is that sexual ornaments are highly variable, more so than non-sexual traits (Price *et al.* 1993). Condition is likely to exhibit high variance so ornamental traits whose expression is strongly condition-dependent are likewise expected to exhibit high variance relative to non-sexual traits whose expression is not, or less, condition-dependent (Pomiankowski & Møller 1995; Rowe & Houle 1996). Comparative analyses of variation, as measured by coefficients of variation (CVs), have supported the hypothesis of increased phenotypic and genetic variance in sexually selected traits (Alatalo *et al.* 1988; Pomiankowski & Møller 1995; Fitzpatrick 1997). However, these general observations need to be extended to cover changes in variation under stress. Under benign conditions, we expect that individual differences in quality will tend to be masked, but that increasing stress will

tend to reveal and amplify these differences. Hence we predict that both phenotypic and genetic variation in sexual ornaments will increase under stress, whilst such changes will be absent (or much reduced) in equivalent non-sexual traits.

These predicted changes in variance can be tested by comparing CVs of sexual and non-sexual traits across environments. But as with trait size, appropriate account needs to be taken of body size. If body size variation increases under stressful conditions, there will inevitably be knock-on increases in variation in other traits. There are two ways in which body size scaling could contribute to higher variation under stress: increase in the allometric slope or increase in the allometric dispersion of traits with body size (Eberhard *et al.* 1998). We need to investigate the relative contribution of these two causes and whether they are greater for sexual ornaments than non-sexual traits.

This chapter describes patterns of condition-dependent trait expression in the stalk-eyed fly *Cyrtodiopsis dalmanni* (Diptera: Diopsidae). Stalk-eyed flies show elongation of the head capsule into long processes ('eye -stalks') onto which the eyes and antennae are laterally displaced. Such hypercephaly is known in several Dipteran families, but Diopsids are unique in that all members, both male and female, possess some degree of eye-stalk elongation (Wilkinson & Dodson 1997; Baker *et al.* 2001). *C. dalmanni* is useful to test the hypotheses discussed above as males have highly exaggerated eyespan compared to females (Figure 3.1), both in absolute and relative terms (David *et al.* 1998). This sexual dimorphism has evolved due to sexual selection. *C. dalmanni* forms nocturnal mating aggregations on root hairs (Burkhardt & de la Motte 1985; Wilkinson & Dodson 1997). Females prefer to roost and mate with males bearing the largest absolute and largest relative eyespan (Wilkinson & Reillo 1994; Hingle *et al.* 2001). In addition, males fight for control of these roosting

sites, and contests are usually won by males with the largest eyespan (Burkhardt & de la Motte 1983, 1987; Wilkinson & Dodson 1997; Panhuis & Wilkinson 1999).

Previously, David *et al.* (1998, 2000) showed that eyespan in male *C. dalmanni* exhibits greater levels of condition dependence than the same measure in females and wing traits in both sexes. However, these two studies are incomplete. Firstly, David *et al.* (1998) used wing size as both a non-sexual trait and a body size index. It is therefore uncertain whether condition dependence arose through changes in the relationship of wing size-to-body size or eyespan-to-body size. David *et al.* (2000) overcame this problem by using thorax length as a measure of body size and looking at the responses of eyespan and wing traits relative to thorax length. However, relative trait size (i.e. dividing by thorax length) does not fully remove the covariance with body size, because traits do not scale isometrically with thorax and exhibit non-zero intercepts (the intercept of male eyespan is more negative than that other traits). Thus the disproportionate change in relative male eyespan may have arisen as an artefact of inappropriate scaling (Packard & Boardman 1999).

In the present study I employed a similar experimental approach to that of David *et al.* (1998), but address the problems highlighted above by using analyses based on General Linear Models (Crawley 1993) with thorax length as a covariate measure of body size. This method has the advantage that it effectively controls for body size, without the drawbacks associated with relative or residual measures (Packard & Boardman 1999; Darlington & Smulders 2001; García-Berthou 2001). My experiment used a broad range of five condition levels and large sample sizes. I carried out comparisons between the male sexual ornament and non-sexual traits, both the homologous trait in females and a wing trait in males. A non-sexual comparison between male and female wing was also undertaken as a control.

I not only measured the response of trait size but also that of trait variation to changes in environmental stress. The analysis tests whether sexual ornaments show heightened condition dependence both in size and variance, and relates these results to the handicap hypothesis of sexual selection.

3.3 MATERIALS AND METHODS

3.3.1 Fly Rearing And Manipulation Of Condition

The laboratory-adapted population of *C. dalmanni* used in this experiment was founded from wild caught flies collected in 1993 from Malaysia by A. Pomiankowski. Flies have since been maintained in cage culture at 25°C on a 12h-light: 12h-dark regime. Population size has been kept high (> 200 individuals) to minimise inbreeding.

Condition was manipulated by varying the amount of food available to developing larvae. Eggs were collected over 24 h periods, and batches of 13 were assigned to one of 5 food levels: 0.015, 0.03, 0.06, 0.09, and 0.12 g puréed corn per egg. The food levels used correspond to the lower regions of a previous experiment (David *et al.* 1998). The food used in this experiment was in a much purer form, so less food produced similar phenotypic effects.

3.3.2 Measurements

Adult flies were collected and frozen every 24 hours. All individuals (268 males and 266 females) were measured later to an accuracy of 0.01 mm using a

monocular microscope and the image analysis program NIH Image (Version 1.55). Measurements were taken of eyespan (between the outermost lateral edges of the eye-bulbs), thorax (middle of the anterior-most part of the head to the posterior edge of the thorax) and wing (branch point of the *MA* and *r-m* veins to the terminus of the RP_4 vein, Gullan & Cranston 1994, p.45, measurement '*x*' in David *et al.* 1998). Wing measurements were taken from one wing per fly, as previous work has shown that larval stress has little or no effect on wing length fluctuating asymmetry (David *et al.* 1998; Bjorksten *et al.* 2000, 2001). Measurements from damaged traits were not recorded, so sample sizes differ. All flies were measured 'blind' by a single person (SC). In the 0.015 g group of males, mean trait sizes and variance were heavily influenced by a single outlier that tested significant in Grubbs' outlier test (eyespan $G = 3.51$, thorax $G = 3.47$, wing $G = 3.33$, all $P < 0.01$, Sokal & Rohlf 1995). This individual was removed from the dataset.

17 replicates of the 5 food treatments were set-up over 11 days. I tested for set-up day effects before pooling. For each trait within each sex we performed an ANOVA on trait size with set-up day as a factor.

3.3.3 Statistical Analysis

Trait size

Food treatment was classified as an ordinal variable (FOOD) and one-way ANOVAs were used to test for significance of FOOD effects on traits within each sex. *t*-tests were used for pair-wise comparisons between adjacent treatments (assuming equal or unequal variances where appropriate) to identify those responsible for significant effects on trait size. Different responses of sexual and non-sexual traits to

food treatment were made in two ways. Eyespan (ES) and wing (Wing) were compared within males in a fixed-effects two-way ANOVA by testing the significance of the TRAIT × FOOD interaction (TRAIT = ES or Wing). The same test was used to compare male and female ES (SEX effects). Comparison between male and female Wing was undertaken as a control.

A significant proportion of the response to stress results from body size scaling (David *et al.* 1998, 2000). The measurement of thorax (THX) was taken *a priori* as a general indicator of body size and included as a covariate in further analyses. General Linear Models (GLMs) were used to examine the effects of food treatment on traits after controlling for body size. I constructed GLMs using three main effects, their interactions, a constant and an error term, based on realistic assumptions and previous work. The three main effects (THX, FOOD, TRAIT), and the THX × TRAIT (i.e. trait allometry) and THX × FOOD interactions had all been shown to be important determinants of trait size in previous work on *C. dalmanni* (David *et al.* 1998). Similarly, the magnitude of condition-dependent changes in allometry was known to vary across traits (David *et al.* 1998), so a FOOD × THX × TRAIT interaction was included if significant (see Trait allometry § below). The remaining second-order interaction (TRAIT × FOOD) was used to detect differences between traits in their response to food treatment after removing the effects of body size. The significance of the TRAIT × FOOD and three-way interactions were determined via *F*-tests on the change in explained variance upon removal of each term from the full model (Crawley 1993, p. 196). The same model was re-run for comparisons between the sexes for ES and Wing traits (TRAIT replaced with SEX factor). Least squares means estimates derived from within-trait GLMs (with factors FOOD, THX and their interaction) were used to visualise changes in trait size with food treatment and pair-

wise *t*-tests were performed between adjacent treatments to infer those responsible for significance of TRAIT × FOOD and SEX × FOOD interactions.

Trait variance

Coefficients of variation ($CV = \sqrt{V}/\overline{X}$) were used to assess changes in the variance of trait size across food treatments. CVs were used to control for differences in trait size. Homogeneity of CVs across food treatments was tested using the Miller-Feltz method (Zar 1996, p. 206), and differences between traits and sexes within each treatment were detected using *Z*-tests compared to critical values from the *t*-distribution with infinite degrees of freedom (Zar 1996, p. 144).

Changes in CVs could in part be due to changes in allometric scaling with body size across food treatments. Allometric slopes of eyespan- and wing-on-thorax were estimated for each sex within each treatment and the response to stress compared across traits. A number of different regression techniques have been proposed, including ordinary least squares (OLS), major axis (MA) and reduced major axis (RMA) regression. Each method has advantages and limitations (see discussions in LaBarbera 1989; Sokal & Rohlf 1995; Eberhard *et al.* 1999; Green 1999). OLS regression is most suited for testing whether eyespan is a more reliable metric of body size variation than other traits because it predicts the expected values of one variable given the other. I therefore employed OLS regression to estimate allometric relationships. In my analysis, I also have been mindful of the potential for slope change to occur simply for artefactual reasons (Pagel & Harvey 1988).

The effect of food treatment on trait allometries was investigated using ANCOVAs and the GLMs described above. I used the FOOD × THX × TRAIT and FOOD × THX × SEX interactions to test the null hypotheses that there were no trait or sex

differences, respectively, in the change of allometric slope with food treatment. I used untransformed trait measures as previous studies in *C. dalmanni* have shown that scaling follows a linear model (Wilkinson & Dodson 1997; David *et al.* 1998; Baker & Wilkinson 2001). The significance of each term was determined by an *F*-test of the change in explained variance as above.

As well as changes in variation due to covariation with body size, there may also be changes in variation due to the trait of interest itself. This effect can be estimated from the distribution around the allometric line. Two analytical methods have been used previously (Eberhard *et al.* 1998). The first, the standard error of the estimate (SEE), uses the square root of the residual mean square of the regression. Since the magnitude of the SEE is proportional to the magnitude of the *Y*-variable, this technique is not appropriate for comparisons across traits, treatments, or sexes. The second method, the allometric dispersion (AD), measures the coefficient of variation that *Y* (i.e. the trait of interest) would have if *X* (i.e. body size) were held constant ($AD = CV_Y(1 - r^2)^{0.5}$), where *r* is the correlation coefficient between *Y* and *X*. This method has the advantage of controlling for differences in the magnitude of the *Y*-variable (via the CV), but is only justified where *r*-values are significant (Eberhard *et al.* 1998). All *r*-values describing correlations between eyespan- or wing-on-thorax were significant. I therefore employed the AD-method for estimating allometric dispersion. I tested for homogeneity of the ADs across treatments to indicate changes in eyespan and wing variation. I then compared the ADs of eyespan and wing within treatments using *Z*-tests as above.

3.3.4 Survival Data

Varying numbers of flies emerged from each treatment. I expected that greater mortality would appear at higher levels of stress. A possible disadvantage of this is that morphological differences between groups were caused by differential survival, rather than from the influence of treatment itself. I therefore tested for differences in survival (the number of flies collected from each batch of 13 eggs) across treatments within sexes.

3.3.5 Adjusting For Multiple Comparisons

In many cases multiple tests were performed on each dataset so the probability of making a type-I error was inflated. I therefore adjusted the significance level using the sequential Bonferroni method (Rice 1989; Sokal & Rohlf 1995). Where correction has important consequences the *P*- values are given using the notation $P_{[\alpha']} = \alpha$, where α' = the adjusted significance level and α = the experiment-wise error rate. To avoid being overly conservative I treated analyses of absolute trait size, body size-controlled trait size, variance, allometry and allometric dispersion separately.

3.4 RESULTS

3.4.1 Set-up Day Effects

No significant variation associated with set-up day was found for eyespan (male $F_{10,237} = 1.52$, female $F_{10,237} = 1.47$, $P = 015$ and $P = 0.13$ respectively.), male thorax ($F_{10,246} = 1.75$, $P = 0.07$) or wing (male $F_{10,248} = 1.30$, female $F_{10,204} = 1.85$, both $P = 0.07$ and $P = 0.06$ respectively) measurements. Significant set-up day effects were found for female thorax measurements ($F_{10,241} = 2.41$, $P = 0.01$), but these were small and showed no pattern. I therefore pooled data from all 11 days.

3.4.2 Trait Size

In environments amenable to maximal growth (0.12 g per egg), *C. dalmanni* males were larger than females for all absolute traits (Figure 3.1; Table 3.1; ES $t_{112} = 55.82$, Wing $t_{117} = 11.83$, Thorax $t_{116} = 6.97$, all $P < 0.001$).

3.4.3 Condition And Trait Size

As food availability declined, the absolute size of all traits decreased in each sex (Figure 3.2; $F_{4,247\text{-}258} \geq 213.37$, all $P < 0.001$). Pair-wise comparisons between adjacent food treatments revealed that changes in trait size occurred mainly in the 0.03 and 0.015 g corn per egg groups (Figure 3.2). There were significant differences

between traits in the magnitude of their response (male ES *vs.* male Wing FOOD × TRAIT $F_{4,505} = 257.28$, male ES *vs.* female ES FOOD × SEX $F_{4,494} = 93.89$, male Wing *vs.* female Wing FOOD × SEX $F_{4,507} = 16.97$, all $P < 0.001$). The sexual ornament, male ES, declined significantly more with stress (47.4% reduction) than non-sexual traits: either male Wing (27.6%) or female ES (27.7%) (Figure 3.2). There was also a greater response to stress in the male Wing compared to the female Wing (20.3%).

The same comparisons were made after taking account of body size using analysis by GLMs with thorax length as a covariate. Again, the male ornament showed heightened sensitivity as males lost more ES than Wing as stressed increased (Figure 3.3; FOOD × TRAIT $F_{4,493} = 5.16$, $P < 0.001$). Similarly, male ES decreased more than female ES as stress increased (Figure 3.3; FOOD × SEX $F_{4,488} = 3.62$, $P < 0.001$). However, initial differences between male and female Wing in their response to stress were accounted for by scaling with body size, as inclusion of THX in the model abolished the previously significant FOOD × SEX interaction (Figure 3.3; $F_{4,499} = 1.53$, $P = 0.19$).

3.4.4 Trait Variance

In environments amenable to maximal growth (0.12 g per egg), there was some evidence of greater variation in the male sexual ornament, as male ES CV (coefficient of variation) was greater than male Wing CV (Figure 3.4, Table 3.2). However, the CVs of male and female ES did not differ (Figure 3.4, Table 3.2). There was also no difference between male and female Wing CVs (Figure 3.4, Table 3.2).

3.4.5 Condition and Trait Variance

Coefficients of variation of all traits in both sexes were non-homogeneous across food treatments (male ES $\chi^2_4 = 199.68$, male Wing $\chi^2_4 = 125.65$, female ES $\chi^2_4 = 173.71$, female Wing $\chi^2_4 = 124.20$, all $P < 0.001$), and tended to increase with stress (Figure 3.4). Taking account of different food treatments revealed that male ES was more variable than both male Wing (in all 5 treatments) and female ES (in 3 treatments) (Table 3.2). Differences between male and female Wing trait CVs were slight, although significant in some cases (Table 3.2).

Changes in body size variation across food treatments may explain the relationships above, as thorax length CV showed similar non-homogeneity (male Thx $\chi^2_4 = 102.06$, female Thx $\chi^2_4 = 75.14$, both $P < 0.001$) and increased with stress (data not shown). To take account of body size variation I analysed changes in the allometric slope and allometric dispersion (AD) of eyespan- and wing-on-thorax. As has been reported before (Wilkinson & Dodson 1997, Baker & Wilkinson 2001), I found that male ES had a much higher allometric slope than female ES (SEX × THX $F_{1,488} = 163.12$, $P < 0.001$), whereas both sexes had similar Wing allometries (SEX × THX $F_{1,499} = 1.99$, $P = 0.16$). In addition, I found that the allometric slopes of male eyespan-on thorax increased significantly with food stress (Figure 3.5; one-way ANCOVA, FOOD × THX $F_{4,242} = 16.63$, $P < 0.001$). This response was seen for all traits in both sexes (Figure 3.5; one-way ANCOVA, FOOD × THX $F_{4,242\text{-}251} \geq 10.03$, all $P < 0.001$). The response of male ES allometry to stress was not different from that of female ES (FOOD × THX × SEX $F_{4,484} = 1.63$, $P = 0.17$). However, the response of male ES allometry to stress was significantly different to that of male Wing allometry

(FOOD × THX × TRAIT $F_{4,493} = 12.97$, $P < 0.001$). The biological significance of this difference was unclear as the male ES slope became proportionally steeper than the male Wing slope between the 0.06 and 0.03 g groups ($t_{493} = 2.82$, $P = 0.005$), but proportionally more shallow between the 0.03 and 0.015 g groups ($t_{493} = 4.80$, $P < 0.001$). In addition, there was no difference between the sexes in the response of Wing allometries (FOOD × THX × SEX $F_{4,495} = 1.80$, $P = 0.13$). These results suggest that sexual and non-sexual traits do not differ consistently in the way their allometries respond to stress.

I used allometric dispersion (AD) as a measure of trait variance after accounting for variation in body size. The ADs of male eyespan-on-thorax showed significant heterogeneity ($\chi^2_4 = 39.46$, $P < 0.001$) and a highly significant increase with food stress (Figure 3.6). This systematic pattern was not as marked in other traits despite some significant variation among their ADs (Figure 3.6; male Wing $\chi^2_4 = 11.63$, $P_{[0.0125]} = 0.02$, female ES $\chi^2_4 = 12.94$, $P_{[0.0167]} = 0.01$, female Wing $\chi^2_4 = 8.27$, $P_{[0.0125]} = 0.08$). Intra-treatment comparisons confirmed the heightened AD of ornaments in low condition males (Table 3.3). Male ES showed significantly greater variation after controlling for body size than male Wing and female ES (in 5/5 and 3/5 treatments respectively, Table 3.3). Male Wing AD was higher than female Wing AD in 2 treatments, but these differences were slight (Table 3.3).

3.4.6 Survival

Food treatment had a significant effect on the survival of *C. dalmanni* (one-way ANOVA, males $F_{4,80} = 3.49$, $P = 0.01$, females $F_{4,80} = 5.19$, $P = 0.001$). This was due to low survival in the 0.015 g per egg treatment group. When this high stress

category was removed from the analyses, the food treatment effect on survival was abolished (males $F_{3,64} = 1.02$, females $F_{3,64} = 1.03$, both $P = 0.39$). It is possible that non-random death in the 0.015 g per egg group influenced our results. I therefore repeated the analyses omitting all flies from the 0.015 g per egg treatment groups. I found that all the contrasts between male and female ES and Wing were unchanged except one. In the reduced data set, male ES no longer showed a stronger response to stress compared to male Wing after controlling for body size ($F_{3,220} = 0.15$, $P = 0.93$). However, male ES did continue to show a stronger response to stress compared to female ES after controlling for body size ($F_{3,436} = 3.10$, $P = 0.027$), and there was no loss of significance in measures showing that male ES was more variable. Hence I conclude that survival differences do not explain the pattern of greater sensitivity of male ES to stress seen in this study.

3.5 DISCUSSION

The condition-dependent handicap hypothesis predicts that ornaments in males from highly dimorphic species under strong sexual selection show heightened condition-dependent expression (Andersson 1986; Pomiankowski 1987, 1988; Grafen 1990; Iwasa *et al.* 1991; Iwasa & Pomiankowski 1994, 1999). The degree of condition dependence seen in sexual traits should be greater than in other non-sexual traits including the homologous trait in females when present. These predictions are borne out in the species of stalk-eyed fly studied here. Absolute male eyespan in the highly sexually dimorphic *C. dalmanni* decreased sharply as condition deteriorated, whereas this striking decline was not present in female eyespan or wing traits. This greater decline in male eyespan also persisted after controlling for body size.

Previous comparative investigations have indicated that sexual traits are more variable than non-sexual traits - measured using CVs, which controls for trait size variation (Alatalo *et al.* 1988; Pomiankowski & Møller 1995; Fitzpatrick 1997). However, none of these analyses investigated the effects of condition on trait variance. I found that variance increased as condition declined, despite the decrease in mean trait size. This pattern found has been reported before in *Drosophila melanogaster* (e.g. Imasheva *et al.* 1999; Fowler & Whitlock 2002) but not previously for an insect species with an exaggerated sexual ornament. My study showed that the sexual trait, male eyespan, was more variable than other traits and this became significantly more so with stress.

To further investigate increased ornament variance, I analysed the allometric properties (slope and dispersion) of male eyespan-on-body size (i.e. thorax length). Eberhard *et al.* (1998) proposed that these patterns have different biological causes: increased slope reflecting a proportionately greater investment in ornaments in larger animals, and increased dispersion reflecting greater genetic and environmental variation, unrelated to body size, that affect the development of ornaments. The first possibility was an increase in allometric slope as condition declined. My results indicate that the male eyespan slope increased with stress. However, similar increases in allometric slopes were found in all other traits in both sexes. So the changes in slope are generic responses, and cannot explain that greater variance observed in the sexual trait when subject to stress.

It is possible that the increase in regression slope with stress is an artefact (Pagel & Harvey 1988). OLS regression assumes that X (body size in this analysis) is known without error. This is not the case, as inevitably there will be measurement and

sampling error in the estimate of body size. This can cause changes in slope across treatments as can be seen from the OLS regression equation,

$$b = \frac{Cov_{XY}}{(Var_X + Var_{error})}$$

If we assume that the covariance of X and Y (Cov_{XY}) scales with the true variance in X (Var_X) but the error variance is approximately constant (Var_{error}), the regression slope (b) will increase with the magnitude of the variance in X. This is the pattern we observed, namely an increase in variance and slope under more stressful conditions. Similar problems occur in MA and RMA regression analyses (Harvey & Pagel 1988). To calculate the extent whether this is the explanation of the changes in variance requires a good estimate of Var_{error}, which I do not have for this study.

The second possibility is an increase in male eyespan variance with food stress beyond that predicted by change in body size variation. I tested for this by measuring the dispersion around the allometric line as condition declined. I found that the male sexual ornament became proportionately more variable with stress after removing the covariance with body size. Stress magnified the variation in eyespan from that predicted by the allometric scaling function. This pattern was much weaker or absent in other traits.

It is noteworthy that food stress reduced the power of eyespan to predict body size. This result suggests that assessment of male eyespan in *C. dalmanni* did not evolve *specifically* to assess body size. So body size is expected to play a limited role in female mate choice or the resolution of fights. Indeed, females are reported to prefer males with large eyespans when body size is controlled for (Wilkinson & Reillo 1994), and experimental studies have indicated that eyespan, independent of

body size or mass, is the chief determinant of contest outcome between male *C. dalmanni* (Panhuis & Wilkinson 1999).

In conclusion, I showed that exaggerated male ornament expression is highly dependent on the condition of the bearer. I also demonstrated that male ornament size became proportionally more variable under stress, and this was attributable to a condition-dependent increase in variance beyond that predicted by body size variation. These responses are distinct to the sexual ornament. Comparable non-sexual traits show much weaker condition dependence in size and variance that is largely accounted for by body size. Thus in *C. dalmanni,* male eyespan provides additional information over and above that given by body size about male condition. These results are consistent with theoretical predictions from condition-dependent handicap models of ornament evolution.

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Table 3.1. Absolute (mm) trait sizes (mean ± SD (*n*)) of flies grown in each environment.

Sex	Food treatment (g corn per egg)	Eyespan	Wing	Thorax
Male	0.015	4.52 ± 0.66 (32)	1.81 ± 0.14 (32)	2.04 ± 0.17 (32)
	0.03	6.48 ± 0.99 (56)	2.13 ± 0.15 (58)	2.48 ± 0.22 (57)
	0.06	8.43 ± 0.36 (47)	2.46 ± 0.07 (50)	2.96 ± 0.12 (50)
	0.09	8.68 ± 0.42 (64)	2.48 ± 0.08 (66)	3.03 ± 0.12 (66)
	0.12	8.60 ± 0.31 (53)	2.50 ± 0.07 (57)	3.03 ± 0.09 (56)
Female	0.015	4.27 ± 0.48 (23)	1.88 ± 0.15 (23)	2.18 ± 0.20 (23)
	0.03	4.99 ± 0.42 (49)	2.09 ± 0.12 (49)	2.48 ± 0.17 (49)
	0.06	5.83 ± 0.19 (68)	2.31 ± 0.06 (69)	2.83 ± 0.14 (70)
	0.09	5.97 ± 0.16 (51)	2.35 ± 0.06 (51)	2.93 ± 0.09 (52)
	0.12	5.91 ± 0.20 (61)	2.36 ± 0.06 (62)	2.91 ± 0.10 (62)

Table 3.2. *Z*-values for between trait comparisons of coefficients of variation (CV).

For CVs refer to **Figure 3.4.**

Treatment	Male ES vs. Male Wing	Male ES vs. Female ES	Male Wing vs. Female Wing
0.015 g	4.59 $P < 0.001$	1.77 $P = 0.08$	0.04 $P = 0.97$
0.03 g	7.42 $P < 0.001$	5.50 $P < 0.001$	2.67 $P = 0.008$
0.06 g	4.23 $P < 0.001$	3.21 $P = 0.001$	0.15 $P = 0.88$
0.09 g	5.00 $P < 0.001$	5.59 $P < 0.001$	2.53 $P = 0.01$
0.12 g	3.03 $P = 0.002$	0.82 $P = 0.41$	1.47 $P = 0.14$

Table 3.3. *Z*-values for between trait comparisons of allometric dispersion (AD). For AD-values refer to **Figure 3.6**.

Treatment	Male ES vs. Male Wing	Male ES vs. Female ES	Male Wing vs. Female Wing
0.015 g	4.67 $P < 0.001$	4.09 $P < 0.001$	0.85 $P = 0.40$
0.03 g	4.83 $P < 0.001$	3.95 $P = 0.001$	2.65 $P = 0.008$
0.06 g	2.68 $P = 0.007$	1.33 $P = 0.18$	0.57 $P = 0.60$
0.09 g	1.97 $P = 0.049$	5.47 $P < 0.001$	3.84 $P = 0.001$
0.12 g	3.01 $P = 0.003$	0.24 $P = 0.81$	0.23 $P = 0.81$

Figure 3.1. Silhouettes of male and female *Cyrtodiopsis dalmanni*. For trait sizes refer to **Table 3.1**.

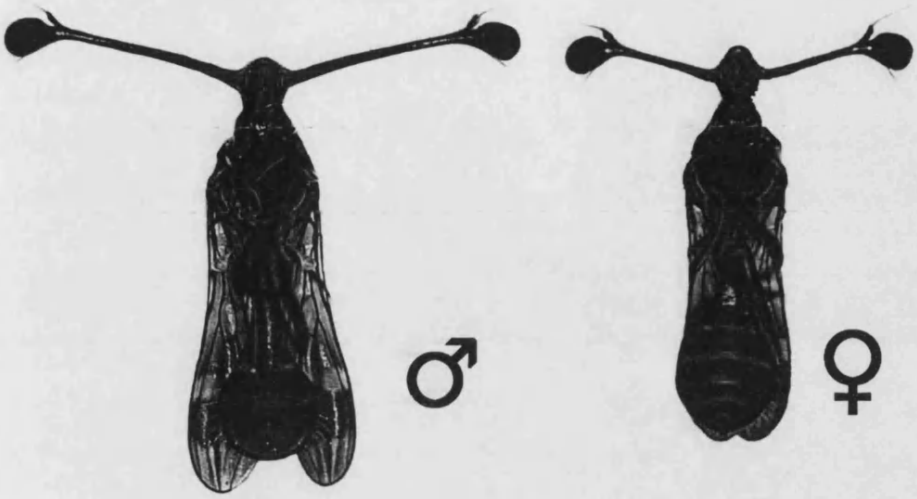


Figure 3.2. Changes in mean eyespan (ES) and Wing traits in response to food treatment in *C. dalmanni*. Trait means were standardised to unity in the 0.12 g treatment group to ease comparisons between different sized traits. Trait sizes from other treatments are expressed as proportions of the standardised 0.12 g groups. Error bars are omitted for clarity. Asterisks denote significance of within-trait, between adjacent treatment comparisons after sequential Bonferroni correction: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

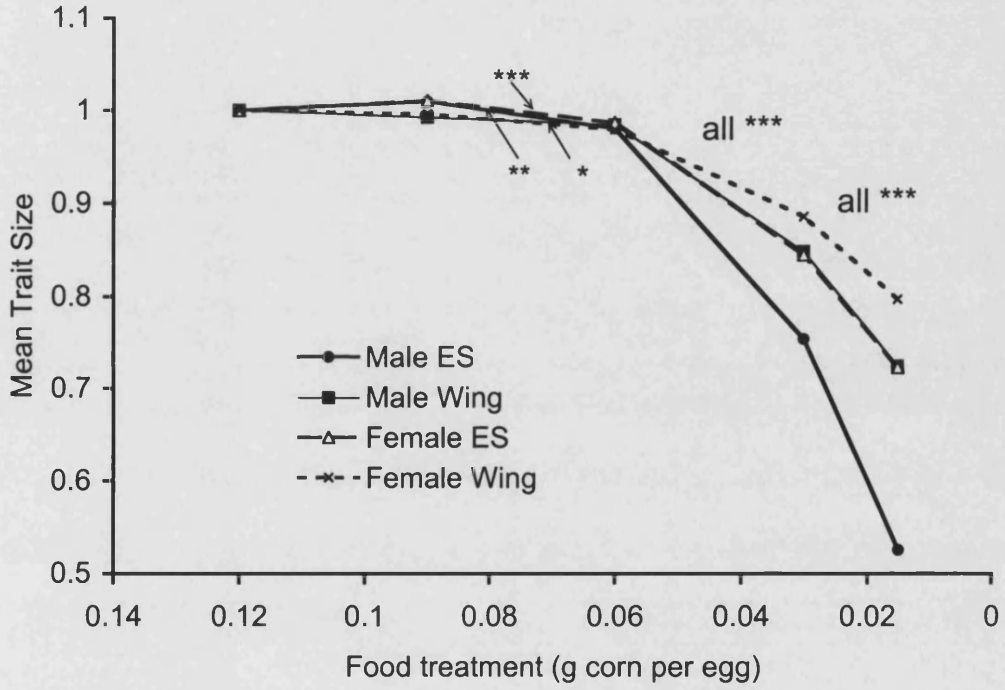


Figure 3.3. Comparisons between traits in their responses to food treatment after taking account of the effects of body size scaling. Least squares means were standardised to unity in the 0.12 g treatment group to ease comparisons between different sized traits. Least squares means from other treatments are expressed as proportions of the standardised 0.12 g groups. Error bars are omitted for clarity. Asterisks indicate significant differences between adjacent treatments after sequential Bonferroni correction: ** $P < 0.01$, * $P < 0.05$.

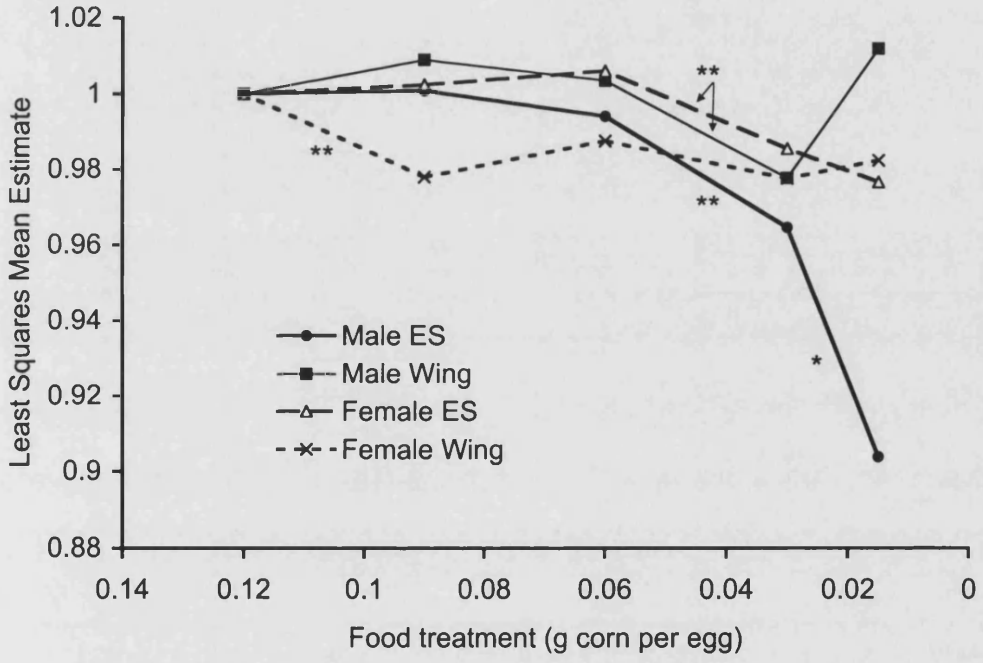


Figure 3.4. Changes in Coefficients of Variation (CVs) (± S.E.) with food treatment. Asterisks indicate significant differences between CVs in adjacent treatments: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ after sequential Bonferroni correction.

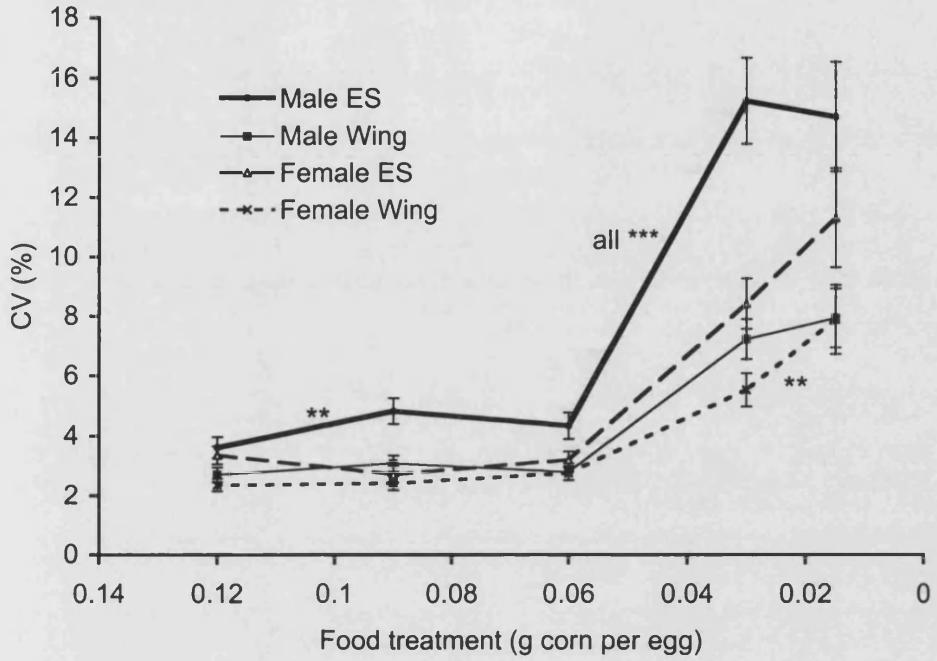


Figure 3.5. Changes in the allometric slope (± SE) of eyespan- and wing-on-thorax with food treatment. Asterisks indicate significant differences between slopes in adjacent treatments: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ after sequential Bonferroni correction.

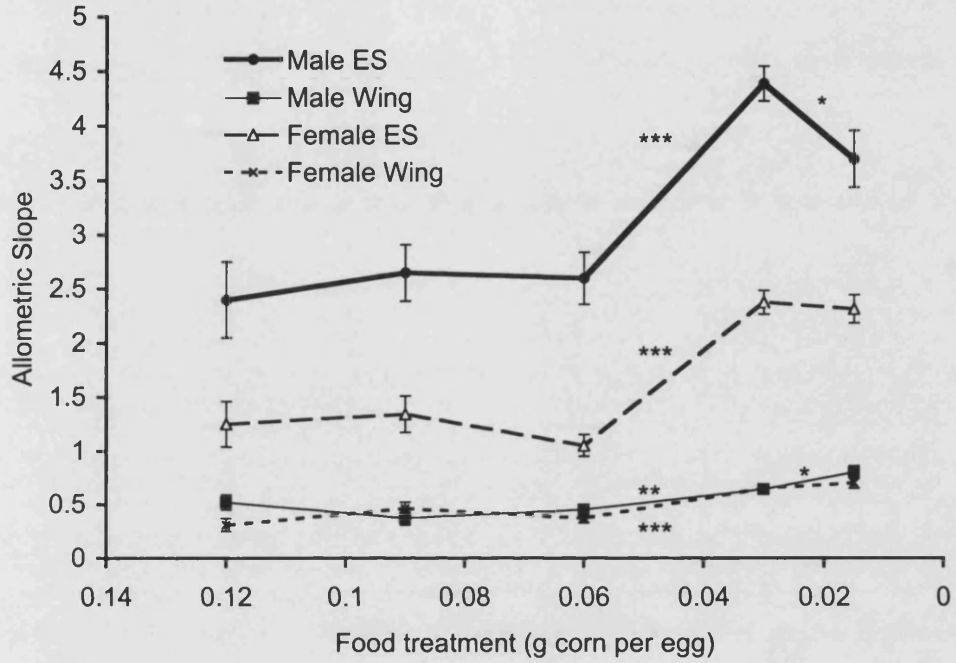
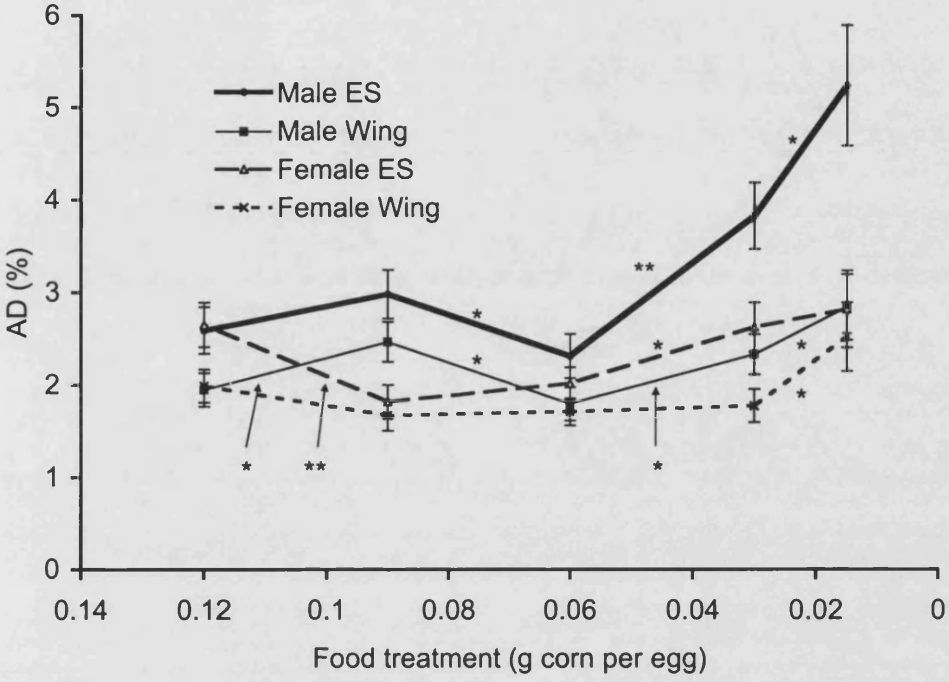


Figure 3.6. Changes in Allometric Dispersion (AD) (± SE) with food treatment. Asterisks denote significant differences between ADs in adjacent treatments: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ after sequential Bonferroni correction.



4

Heightened Condition Dependence Is Not A General Feature Of Male Eyespan In Stalk-Eyed Flies (Diptera: Diopsidae)

4.1 ABSTRACT:

Stalk-eyed flies are exemplars of sexual selection leading to the evolution of exaggerated male ornaments (eyespan). In *Sphyracephala beccarri*, males are subject to little or no sexual selection through female mate choice and there are only minor sex differences in eyespan. I used *S. beccarri* to test whether male eyespan only evolves heightened condition dependence when it becomes sexually exaggerated as predicted by handicap models of sexual selection. Male eyespan was found to show heightened condition dependence compared to a control trait (wing length) when under food stress, using both absolute and body size-controlled measurements. However, female eyespan showed a similar pattern of greater sensitivity, and there was no difference between the sexes in the degree of increased eyespan sensitivity. I compared the response to stress in *S. beccarri* with that in *Cyrtodiopsis dalmanni*, a highly dimorphic stalk-eyed fly species under strong sexual selection, to demonstrate the positive association of heightened condition dependence with traits that have become exaggerated through sexual selection. The finding in *S. beccarri* that eyespan is a more sensitive indicator of condition than other traits even in an unexaggerated state, suggests that this may have acted as a pre-adaptation to its role in sexual signalling in other Diopsid species. This result accords with Fisher's original view of how sexual selection is initiated.

4.2 INTRODUCTION

Handicap models of sexual selection propose that females use male sexual ornaments to assess male genetic quality, in order to increase the fitness of offspring (Andersson 1986; Pomiankowski 1987, 1988; Grafen 1990; Iwasa *et al.* 1991; Iwasa & Pomiankowski 1994). Exaggerated sexual traits are assumed to reduce viability, so only males in good condition can bear the cost of an extravagant ornament. Consequently, a key prediction of the handicap hypothesis is that exaggerated male ornaments should have evolved heightened condition-dependent expression.

A number of reviews have concluded that condition dependence of male ornaments is widespread (Andersson 1994; Johnstone 1995). However, this view is less well founded than suggested, because it is based largely on correlational data. There are relatively few experimental studies, and many of these have not employed appropriate controls (reviewed in Cotton *et al.* 2004a; Chapter 2). For instance, most experiments failed to compare the sexual trait with non-sexually selected control traits and it is not possible to assess whether traits possess enhanced condition dependence as required by the handicap hypothesis. In addition, many experiments have only investigated the effect of extreme differences in environmental condition (i.e. no *vs.* extreme stress), neither of which may represent conditions typically experienced in nature. There remains a pressing need for more appropriately designed and controlled experiments to test the generality of heightened condition dependence in sexually selected characters.

Male eyespan is well known as a trait in stalk-eyed flies that is subject to sexual selection primarily through female choice (Wilkinson & Dodson 1997), and has become repeatedly exaggerated in several Diopsid lineages (Baker & Wilkinson

2001). I have previously worked on *Cyrtodiopsis dalmanni*, a stalk-eyed fly species that is highly sexually dimorphic for eyespan (Cotton *et al.* 2004b, Chapter 3). This species forms nocturnal mating aggregations that are controlled by large eyespan males (Wilkinson & Dodson 1997; Burkhardt & de la Motte 1985). Females prefer to roost and mate with males with larger eyespan (Wilkinson & Reillo 1994; Hingle *et al.* 2001). I have shown that male eyespan in *C. dalmanni* exhibits heightened levels of condition dependence after controlling for body size than seen in female eyespan or wing length in either sex (Cotton *et al.* 2004b; Chapter 3; see also David *et al.* 1998). In addition, there is evidence of a genetic basis in the response of male eyespan to environmental variation in condition; some genotypes produced large male eyespan in all environments, whilst others became progressively smaller as stress increased (David *et al.* 2000; see also Chapter 5).

In this paper I take a new approach by considering condition dependence of male eyespan in the Diopsid stalk-eyed fly *Sphyracephala beccarri*. In contrast to *C. dalmanni*, it is a species with only slight sexual dimorphism for eyespan (Baker & Wilkinson 2001). There are no field observations for this species, but in the laboratory *S. beccarri* does not form nocturnal mating aggregations. Individuals mate opportunistically and males exhibit post-copulatory passive mate guarding (S. Cotton *personal observation*), which has also been recorded in a closely related species, *S. brevicornis* (Hochberg-Stasny 1985). There is no evidence of female mate choice for male eyespan in this or related sexually monomorphic species (Wilkinson & Dodson 1997; Wilkinson *et al.* 1998).

My objective was to use *S. beccarri* to assess the condition dependence of male eyespan in a species subject to little or greatly reduced sexual selection. Larvae were subjected to 5 levels of food treatment, varying from low (abundant food) to

high (minimal food) larval stress, using the same protocol developed to study the sexually dimorphic species *C. dalmanni*. I compared male eyespan with two non-sexual control traits: male wing length and female eyespan. I also made a control comparison between female eyespan and female wing length. The analysis was carried out on absolute trait size and on measurements controlled for body size variation. This allowed me to test the assumption that in the absence of an evolved sexual signalling function, male eyespan responds to stressful conditions in the same way as other non-sexual traits. That is, I expect that traits free from sexual selection to show no heightened condition dependence. I complete my analysis of *S. beccarri* by comparing its response with those of *C. dalmanni* flies that were subjected to the same food treatments in a previous study (Cotton *et al.* 2004b; Chapter 3).

4.3 MATERIALS & METHODS

4.3.1 Fly Rearing

The laboratory-adapted population of *S. beccarri* used in this experiment was transferred to the lab in 1999, after being collected from South Africa in 1993 by Jerry Wilkinson (University of Maryland). It has since been maintained in cage culture at 25°C on a 12h-light: 12h-dark photoperiod, and population sizes have been kept high (> 200 individuals) to minimise inbreeding.

Condition was manipulated by rearing larvae under varying degrees of food stress. Eggs were collected from stock populations over 24 h periods, and batches of 13 were assigned to one of 5 food treatments: 0.015, 0.03, 0.06, 0.09, and 0.12 g

homogenised corn per egg. These food levels were chosen as pilot work showed they caused significant phenotypic changes in trait size (S. Cotton *unpublished data*).

4.3.2 Measurements

Adult flies were collected and frozen every 24 hours. All individuals (237 male and 271 female) were measured later to an accuracy of 0.01 mm using a monocular microscope and the image analysis program NIH Image (Version 1.55). Measurements were taken of eyespan (between the outermost lateral edges of the eye-bulbs), thorax (middle of the anterior-most part of the head to the posterior edge of the thorax) and wing length (the branch point of the *MA* and *r-m* veins to the terminus of the RP_4 vein, p.45 in Gullan & Cranston (1994), measurement '*x*' in David *et al.* (1998)). Measurements from damaged traits were not recorded, so sample sizes differ. All flies were measured 'blind' by a single person (SC).

4.3.3 Statistical Analysis

Differences between the absolute sizes of traits in *S. beccarri* were determined with *t*-tests by comparing flies reared under benign environments (0.12 g per egg). Food treatment was classified as an ordinal variable (FOOD) and one-way ANOVAs were used to test for significance of FOOD effects on traits within each sex. Comparisons between adjacent treatments were performed to identify those responsible for significant effects on trait size. Eyespan (ES) and wing length were compared within each sex in a General Linear Model (GLM) (with factors FOOD, TRAIT and FOOD × TRAIT) to detect differences between traits (TRAIT = ES or wing

length) in their response to food treatment. The significance of the interactions was determined via *F*-tests on the change in explained variance upon removal of each term from the full model (Crawley 1993, p. 196). Treatments responsible for significant interactions were identified using pair-wise ordinal FOOD × TRAIT terms derived from the model. Similar analyses were performed to compare the response of traits across sexes (GLM with factors FOOD, SEX and FOOD × SEX). To investigate whether differences between the response of ES and wing length to food stress varied across sexes I tested the significance of the SEX × FOOD × TRAIT interaction in a GLM containing all lower order interactions and main effects.

A significant proportion of the response to stress is likely to result from body size scaling (David *et al.* 1998, 2000; Cotton *et al.* 2004b; Chapter 3). The measurement of thorax (THX) was therefore taken *a priori* as a general indicator of body size and included as a covariate in further analyses. Sexual dimorphism in trait size after controlling for body size in the benign food treatment (0.12 g corn per egg group) was determined using a GLM with factors THX, SEX and THX × SEX.

To assess the effect of food treatment I constructed GLMs using three main effects (FOOD, TRAIT, THX) and their interactions (if significant or required). This model was based on previous work with *C. dalmanni* (David *et al.* 1998; Cotton *et al.* 2004b; Chapter 3). The second-order FOOD × TRAIT interaction was used to detect differences between male ES and wing length in their response to food treatment after removing the effects of body size. This analysis was repeated in females and for separate comparisons of ES and wing length across sexes by replacing TRAIT effects with SEX effects. Allometric slopes differ between traits and sexes, and this makes Least Squares Mean estimates (LSMs) generated from covariance models containing both FOOD and TRAIT or SEX terms difficult to compare. So, in order to visualise the

nature of significant FOOD × TRAIT or FOOD × SEX interactions (i.e. the differences between traits and sexes in their response to food treatment) I plotted the LSMs estimated from within-trait GLMs (with factors FOOD, THX and their interaction). Ordinal between-treatment contrasts from within-trait GLMs were used to identify those responsible for changes in body size-adjusted trait size. As with absolute trait size, I tested the null model that the difference between ES and wing length responses was the same in males and females after controlling for body size through the SEX × FOOD × TRAIT interaction in a GLM containing SEX, FOOD and TRAIT main effects, THX as a covariate and all (significant or required) interactions.

4.3.4 Comparison With *C. dalmanni*

I compared the response of *S. beccarri* to food stress with that observed in *C. dalmanni*, a highly sexually dimorphic species. Eggs were collected from a laboratory-adapted population of *C. dalmanni* that originated from flies collected in Malaysia by A. Pomiankowski in 1993. Larvae were exposed to the same 5 food treatments. Only brief results are given (sample size 267 male and 266 female); for a more detailed analysis of *C. dalmanni* see Chapter 3 (see also Cotton *et al.* 2004b). Differences between the two species in trait size sexual dimorphism were examined using the interaction between SEX and TRAIT effects in two-factor ANOVAs.

4.3.5 Adjustment For Multiple Comparisons

As multiple tests of the effect of food treatment were performed on each trait, I adjusted the significance level using the sequential Bonferroni method (Rice, 1989;

Sokal & Rohlf, 1995). To avoid being overly conservative I treated analyses of the response to food treatment of absolute trait size and body size-controlled trait size separately within each species.

4.4 RESULTS

4.4.1 Trait Size

In the environment amenable to maximal growth (0.12 g per egg), female body size in *S. beccarri* was larger than male body size, using thorax length as an estimate of body size (Figure 4.1, Table 4.1). Wing length was also larger in females, and so to a minor extent was eyespan (Figure 4.1, Table 4.1). To assess male and female differences in wing length and eyespan independent of body size, I entered thorax length as a covariate in General Linear Models (GLMs), and looked for sex differences. After taking account of body size differences between the sexes, females still showed larger wing length, but eyespan was greater in males (Table 4.1).

4.4.2 Condition Dependence

Food treatment had significant effects on the absolute size of all traits; flies became smaller as food availability declined (Figure 4.2; $F_{4,218\text{-}252} \geq 68.45$, all $P < 0.001$). Changes in trait size became greater as food stress increased and were particularly marked in the two most stressful treatments (0.03 and 0.015 g corn per egg; Figure 4.2).

I tested whether male eyespan was more sensitive to stress than other traits. Absolute male eyespan was more sensitive to food stress than male wing length (Figure 4.2; FOOD × TRAIT $F_{4,436} = 20.30$, $P < 0.001$). This difference occurred in the two most stressful treatments (Figure 4.2; 0.06 – 0.03 g × TRAIT $t = 3.19$, $P = 0.002$, 0.03 – 0.015 g × TRAIT $t = 3.51$, $P < 0.001$). However, male and female eyespan did not differ in their response to food treatment (Figure 4.2; FOOD × SEX $F4,470 = 1.70$, $P = 0.15$).

I then tested the response of absolute female eyespan and found it was more sensitive than female wing length (Figure 4.2; FOOD × TRAIT $F_{4,502} = 17.25$ $P < 0.001$). This difference was also limited to the two most stressful groups (Figure 4.2; 0.06 – 0.03 g × TRAIT $t = 3.14$, $P = 0.002$, 0.03 – 0.015 g × TRAIT $t = 3.80$, $P < 0.001$). An explicit comparison of the sexes showed that the different response of absolute eyespan and wing length was similar in both males and females (SEX × FOOD × TRAIT $F_{4,938} = 0.31$, $P = 0.87$). So it appears from absolute measures that eyespan is a more sensitive trait than wing length, but there is no evidence of heightened sensitivity attributable to *male* eyespan.

As body size differed between the sexes and responded to food stress, the patterns reported above could be due to changes in body size. Female thorax size responded more to stress than male thorax size (FOOD × SEX $F_{4,470} = 8.00$, $P < 0.001$, data not shown). So I repeated the analyses with thorax length as a covariate in GLMs. After adjusting for body size, food treatment still had significant effects on all traits (FOOD $F_{4,212\text{-}257} \geq 4.37$, all $P \leq 0.002$). Male eyespan remained more sensitive to food treatment than male wing length after controlling for body size variation (Figure 4.3; FOOD × TRAIT $F_{4,424} = 5.13$, $P < 0.001$). In addition, male eyespan was now more

sensitive than female eyespan after controlling for body size (Figure 4.3; FOOD × SEX $F_{4,459} = 8.63$, $P < 0.001$).

I found a similar trend towards female eyespan being more sensitive than female wing length after controlling for body size variation (Figure 4.3; FOOD × TRAIT $F_{4,496} = 2.14$, $P = 0.075$). Comparison of the sexes showed that the different response of eyespan and wing length was similar in males and females when body size differences were taken into account (THX in model: SEX × FOOD × TRAIT $F_{4,920} = 1.22$, $P = 0.30$). This confirms the result found with absolute trait size, that the heightened response of eyespan to food treatment was a general rather than sex-specific feature.

I next investigated the possibility that general sex differences could account for these patterns. After controlling for body size, male wing length was more sensitive to food stress than female wing length (Figure 4.3; FOOD × SEX $F_{4,462} = 12.51$, $P < 0.001$) and male eyespan was more sensitive than female eyespan (see above). These relationships hint at a greater sensitivity of male traits in general. However, such a male effect was not seen with absolute trait measures. The reverse pattern occurred as absolute female wing length was more sensitive to food stress than male wing length (Figure 4.2; FOOD × SEX $F_{4,468} = 7.08$, $P < 0.001$), and there was no difference between the sexes in eyespan sensitivity (see above). The lack of a consistent pattern in absolute and body size-controlled comparisons leads me to exclude the hypothesis of generally heightened *male* sensitivity to food stress.

4.4.3 Comparison With *C. dalmanni*

In the most benign environment (0.12 g per egg), all male *C. dalmanni* traits were larger than in females (Figure 4.1, Table 4.1). After controlling for body size,

both male eyespan and wing length remained larger (Table 4.1). Inter-specific comparisons of trait size in the 0.12 g treatment group revealed that absolute trait size sexual dimorphism was greater in *C. dalmanni* than *S. beccarri* for all characters but most markedly for eyespan, both before (SPECIES × SEX; thorax $F_{1,196} = 229.26$, eyespan $F_{1,191} = 2181.74$, wing length $F_{1,196} = 427.95$, all $P < 0.001$) and after adjusting for body size (SPECIES × SEX; eyespan $F_{1,187} = 95.13$, wing length $F_{1,191} = 12.31$, both $P < 0.001$).

As food availability declined, the absolute size of eyespan and wing length decreased in *C. dalmanni* ($F_{4,247\text{-}258} \geq 213.37$, all $P < 0.001$). As has been reported elsewhere, traits differed in the magnitude of their response to food stress (Cotton *et al.* 2004b; Chapter 3). Male absolute eyespan declined more than male wing length (Figure 4.4; FOOD × TRAIT $F_{4,505} = 257.28$, $P < 0.001$) and female eyespan (Figure 4.4; FOOD × SEX $F_{4,494} = 93.89$, $P < 0.001$). Absolute female eyespan also responded more than female wing length (Figure 4.4; $F_{4,496} = 112.74$, $P < 0.001$). However, unlike *S. beccarri*, there was a sex difference in the degree of increased eyespan sensitivity when compared to wing length (SEX × FOOD × TRAIT $F_{4,1001} = 76.83$, $P < 0.001$), demonstrating that absolute male eyespan exhibits heightened condition dependence.

Similar patterns were observed when thorax length was included in GLMs (Cotton *et al.* 2004b; Chapter 3). After controlling for body size, the exaggerated male ornament in *C. dalmanni* was more sensitive to larval stress than male wing length (Figure 4.5; FOOD × TRAIT $F_{4,493} = 5.16$, $P < 0.001$) or female eyespan (Figure 4.5; FOOD × SEX $F_{4,488} = 3.62$, $P < 0.001$). A similar trend was found in females, with body size-controlled eyespan responding more than female wing length (Figure 4.5; FOOD × TRAIT $F_{4,486} = 2.20$, $P = 0.068$). However, again unlike *S. beccarri*, the heightened

condition-dependence of eyespan compared to wing length in males remained after controlling for body size (SEX × FOOD × TRAIT $F_{4,987} = 2.81$, $P = 0.02$).

4.5 DISCUSSION

The co-evolution of female preference for condition-dependent male sexual traits is central to handicap models of sexual selection (Iwasa *et al.* 1991; Iwasa & Pomainkowski 1994, 1999). Theory predicts that as male ornaments become exaggerated (and therefore costly), their expression becomes more closely dependent on the quality of the bearer as only males in good condition can afford to pay the cost of a large ornament. This leads to the prediction that the condition dependence of male ornaments will covary positively with the strength of sexual selection and their degree of exaggeration.

I previously tested this prediction in *C. dalmanni*, a stalk-eyed fly species with greatly exaggerated male eyespan. In *C. dalmanni*, male eyespan shows heightened condition dependence relative to female eyespan and male wing length, both before and after controlling for body size variation (Cotton *et al.* 2004b; Chapter 3; see also David *et al.* 1998).

In this paper I adopted the alternative approach and tested the overlooked assumption that male eyespan in species subject to little or no sexual selection should not be strongly condition-dependent. Using the weakly dimorphic stalk-eyed fly species *S. beccarri*, I found that male eyespan was more sensitive than male wing length to changes in food conditions, using both absolute and body size-controlled measurements. At first sight this argues for heightened sensitivity in male eyespan. However, female eyespan showed a similar pattern of greater sensitivity to changes in

condition compared to female wing length, again using absolute and body size-controlled measurements. There was no difference between the sexes in the degree of increased sensitivity of eyespan compared to wing length. So I uncovered a *trait* difference, not a sex-specific trait difference.

These results were unexpected but they are in line with the hypothesis that the lack of sexual selection in *S. beccarri*, and the absence of sexual eyespan exaggeration, has not resulted in heightened condition dependence of male eyespan. They suggest why mate choice based on eyespan exaggeration may have originally evolved in the Diopsidae. Fisher (1915, 1930) proposed that sexual selection would be initiated if female preference arose for male traits that conferred a natural selection advantage. Absolute and body size-controlled eyespan fulfil this criterion for sexual selection targets, as they are more sensitive indicators of larval food stress even in their non-exaggerated state. So a female preferring males with larger absolute and/or body-sized controlled eyespan would on average mate with males in better condition. Assuming that in nature there is a genetic component to condition and the response to environmental stresses (like food limitation), such female preference would result in inherited fitness benefits. My work suggests that these benefits would be greater for mate choice based on eyespan than on other traits.

This hypothesis needs to be investigated further. I only compared eyespan to one other trait (wing length). More contrasts are needed to more firmly establish that eyespan is a more sensitive trait; it remains possible that eyespan reacts like other traits whilst wing length is an insensitive trait. It might also be revealing to analyse more closely related species of *S. beccarri*. Within the *Sphyracephela* group are species like *S. bipunctipennis* that has evolved greater exaggeration of male eyespan and marked sexual dimorphism, and *S. brevicornis* which has evolved sexual

monomorphism for eyespan allometry as well as for absolute eyespan (Baker & Wilkinson 2001).

Another finding in this study was that body size-adjusted male eyespan was more sensitive to food stress than the homologous female trait. The same was true for body size-adjusted male wing length compared to the homologous female trait. This implies that male traits are more sensitive to stress than those of females. However this hypothesis is not supported by the comparison of absolute trait values, which are not sexually different for eyespan sensitivity and show greater female sensitivity for wing length. This disparity between absolute and body size-adjusted trait measures does not support the hypothesis of generally heightened *male* sensitivity to food stress.

I compared the responses of *S. beccarri* with those of *C. dalmanni* to test the hypothesis that the condition dependence of male ornaments covaries positively with the strength of sexual selection and the degree of exaggeration. *C. dalmanni* is a highly sexually dimorphic stalk-eyed fly species with greatly exaggerated male eyespan, a trait subject to strong sexual selection. I found that male eyespan in *C. dalmanni* showed heightened condition dependence relative to male wing length and female eyespan, both before and after controlling for body size variation (this Chapter; Cotton *et al.* 2004b; Chapter 3). As in *S. beccarri*, female eyespan also declined with stress in *C. dalmanni*. But unlike *S. beccarri*, the male response in *C. dalmanni* was markedly greater in eyespan relative to wing length both for absolute and body size-controlled measures. Thus I found a positive association between the degree of exaggeration and the degree of condition dependence.

In conclusion, my experiments add weight to the prediction that heightened condition dependence is associated specifically with costly *exaggeration* of male

sexual traits. This finding is consistent with the prediction made by handicap models of sexual selection. In addition, I raise the possibility that eyespan, even in its unexaggerated state, is a more sensitive indicator of condition than other traits, which may have acted as a pre-adaptation to its role in sexual signalling in other Diopsid species. This latter finding accords well with Fisher's (1915, 1930) original discussion of how sexual selection is initiated, but needs to be substantiated by further investigation.

4.6 LITERATURE CITED

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Table 4.1. Absolute (mean ± SD (*n*)) and Least Squares Mean estimates (LSM ± SE) of trait size (mm) of flies grown in benign environments (0.12 g corn per egg).

	Absolute values				LSM estimates			
Species	Trait	Males	Females	Difference	Males	Females	Difference
S. beccarri:							
	Eyespan	2.20 ± 0.05 (37)	2.22 ± 0.05 (44)	$t_{79} = 2.25, P = 0.03$	2.24 ± 0.01	2.15 ± 0.01	$F_{1,77} = 22.32, P < 0.001$
	Wing	1.71 ± 0.04 (37)	1.91 ± 0.05 (44)	$t_{80} = 14.72, P < 0.001$	1.75 ± 0.01	1.87 ± 0.01	$F_{1,78} = 54.65, P < 0.001$
	Thorax	2.06 ± 0.09 (38)	2.33 ± 0.07 (44)	$t_{79} = 19.91, P < 0.001$			
C. dalmanni:							
	Eyespan	8.60 ± 0.31 (53)	5.92 ± 0.20 (61)	$t_{112} = 55.82, P < 0.001$	8.46 ± 0.03	5.98 ± 0.03	$F_{1,110} = 3363.86, P < 0.001$
	Wing	2.50 ± 0.07 (57)	2.36 ± 0.06 (62)	$t_{117} = 11.83, P < 0.001$	2.47 ± 0.01	2.38 ± 0.01	$F_{1,114} = 61.52, P < 0.001$
	Thorax	3.03 ± 0.09 (56)	2.91 ± 0.10 (62)	$t_{116} = 6.97, P < 0.001$			

Figure 4.1. Silhouettes of male and female a) *S. beccarri*, and b) *C. dalmanni*. Scale bars: 2 mm (vertically).

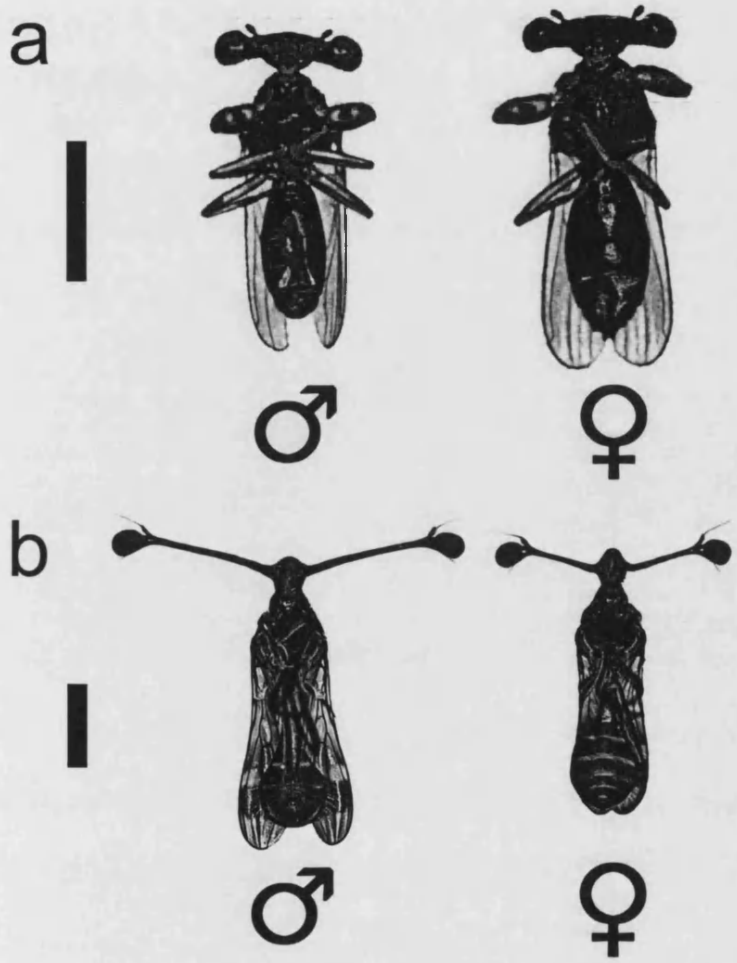


Figure 4.2. Changes in mean eyespan (ES) and wing length of S. *beccarri* in response to food treatment. Trait means were standardised to unity in the 0.12 g treatment group to ease comparisons between different sized traits. Trait sizes from other treatments are expressed as proportions of the standardised 0.12 g groups. Error bars are omitted for clarity. Asterisks denote significance of within-trait, between-adjacent treatment comparisons after sequential Bonferroni correction: *** $P < 0.001$.

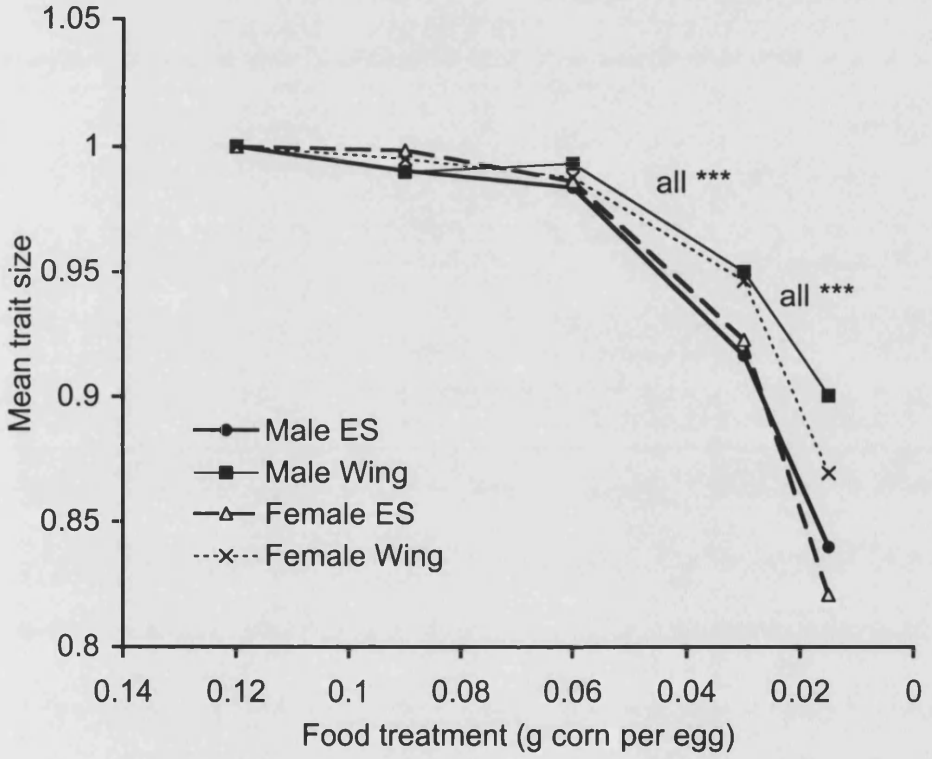


Figure 4.3. Comparisons between eyespan (ES) and wing length of *S. beccarri* in their response to food treatment after controlling for body size. Least squares means estimates were standardised to unity in the 0.12 g treatment group to ease comparisons between different sized traits. Least squares means from other treatments are expressed as proportions of the standardised 0.12 g groups. Error bars are omitted for clarity. Asterisks indicate significance of within-trait, between-adjacent treatment comparisons after sequential Bonferroni correction: *** $P < 0.001$, * $P < 0.05$.

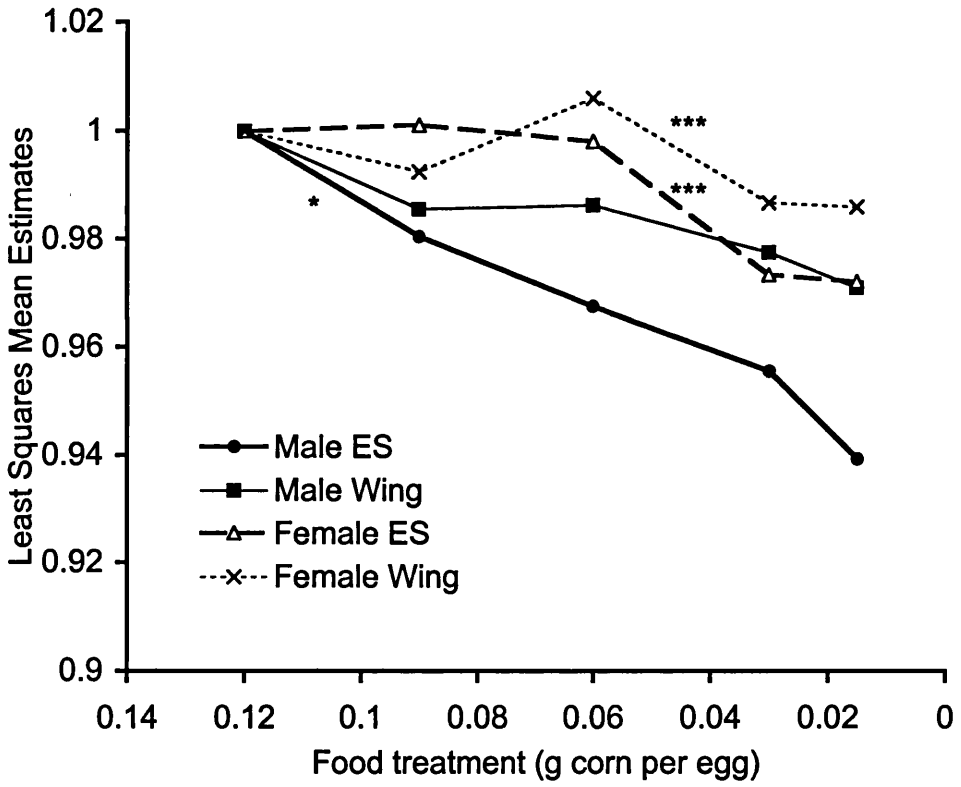


Figure 4.4. Changes in mean eyespan (ES) and wing length of *C. dalmanni* in response to food treatment. Trait means were standardised to unity in the 0.12 g treatment group to ease comparisons between different sized traits. Trait sizes from other treatments are expressed as proportions of the standardised 0.12 g groups. Error bars are omitted for clarity. Asterisks denote significance of within-trait, between-adjacent treatment comparisons after sequential Bonferroni correction: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

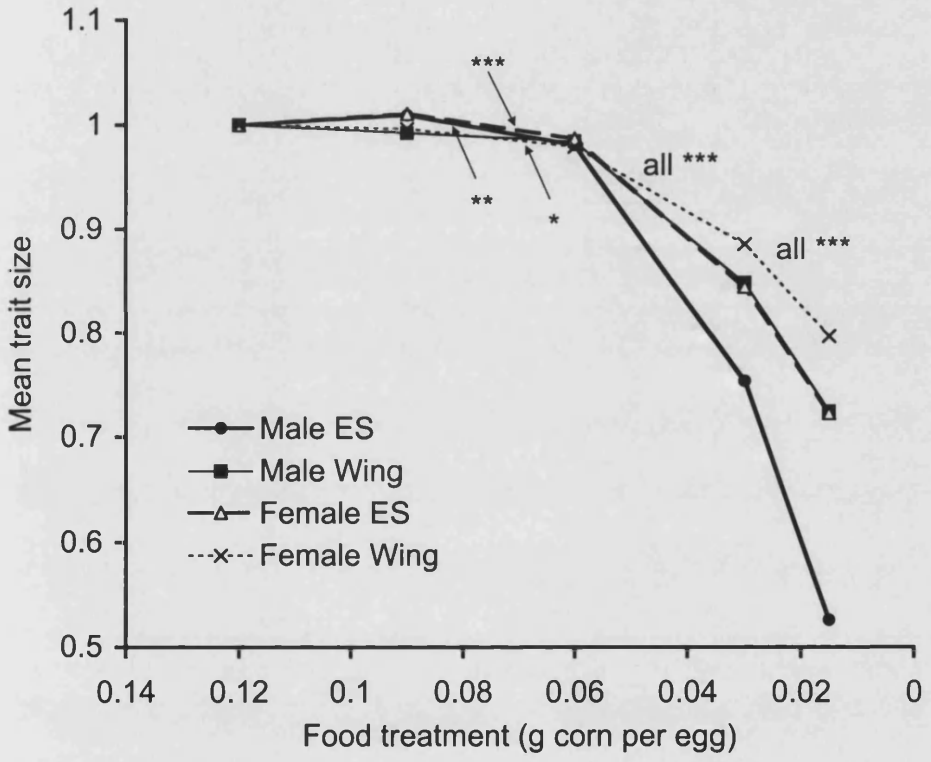
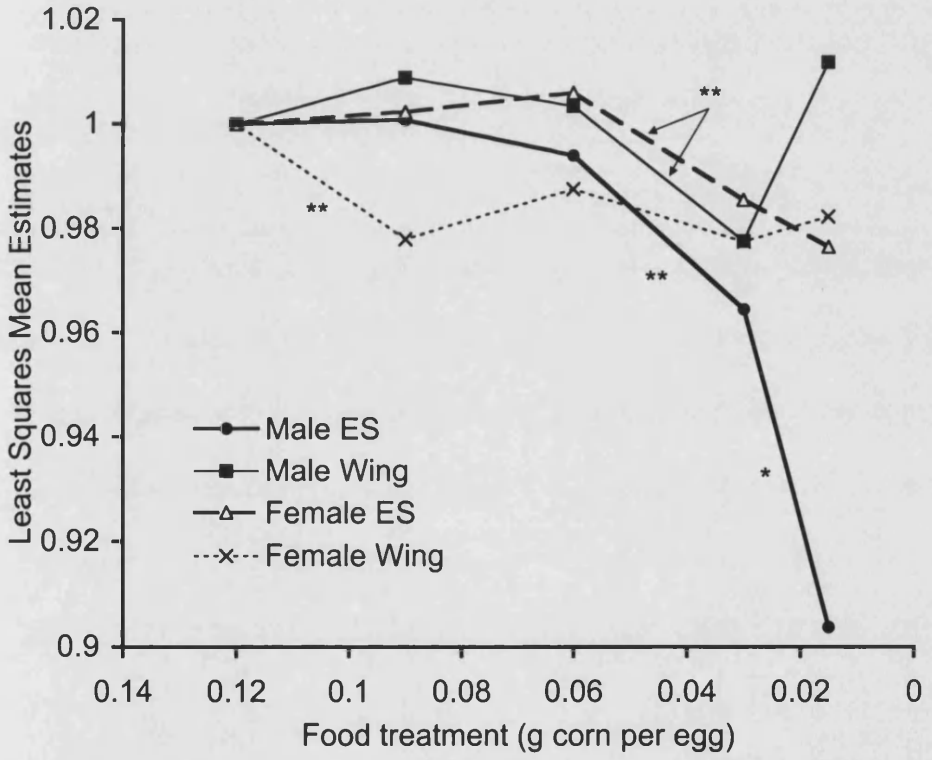


Figure 4.5. Comparisons between eyespan (ES) and wing length of *C. dalmanni* in their response to food treatment after controlling for body size. Least squares means estimates were standardised to unity in the 0.12 g treatment group to ease comparisons between different sized traits. Least squares means from other treatments are expressed as proportions of the standardised 0.12 g groups. Error bars are omitted for clarity. Asterisks indicate significance of within-trait, between-adjacent treatment comparisons after sequential Bonferroni correction: ** $P < 0.01$, * $P < 0.05$.



5

The Genetic Basis Of Heightened Condition-Dependent Ornament Expression In The Stalk-Eyed Fly *Cyrtodiopsis dalmanni* (Diptera: Diopsidae)

5.1 ABSTRACT:

According to the handicap hypothesis of sexual selection, exaggerated male ornaments are costly and signal the genetic quality of their bearer through heightened condition-dependent expression. This assumes a heritable basis to ornament condition dependence. I tested this assumption in the stalk-eyed fly, *Cyrtodiopsis dalmanni.* Males of this species possess greatly exaggerated eyespan, which is under strong sexual selection through female choice. Inbreeding was used to create distinct genotypes, and the genetic basis of ornament condition dependence was investigated by comparing the performance of genotypes (inbred lines) along an experimental gradient of environmental stress. Contrasts were also made between the genetic component of male eyespan expression and that of non-sexual traits, both before and after controlling for body size scaling differences. I found significant interactions between genotype and environmental stress for male eyespan; lines that produced a large ornament in one environment tended to do so in others. Importantly, stress inflated these differences between genotypes leading to an increase in the genetic variance of the male ornament. Such patterns were present to a much lesser extent in non-sexual traits, and persisted before and after controlling for size. Within-line variance was also greater in male eyespan and increased more with stress than other traits. In addition, those lines that performed better under environmental stress showed less increase in variance with stress, which suggests that higher quality genotypes may be more resistant to environmental heterogeneity. These findings accord well with handicap models of sexual selection, and strongly implicate "good genes" as the target and potential benefit of female choice in this species.

5.2 INTRODUCTION

Handicap models of sexual selection propose that male sexual ornaments have evolved to signal male genetic quality (Zahavi 1975; Andersson 1986; Pomiankowski 1987, 1988; Grafen 1990; Iwasa *et al.* 1991; Iwasa & Pomiankowski 1994), so that females mating with the most ornamented males acquire paternally derived fitness benefits for their offspring. Male ornaments are predicted to have co-evolved with female preference to be larger, and so more costly. Central to the handicap hypothesis is the expectation that males in good genetic condition signal their quality through greater sexual trait size or more vigorous display, whilst males in worse condition are unable to do this because they cannot bear the viability costs associated with such extravagance. This leads to the prediction of heightened condition-dependent expression of sexual ornaments (Iwasa *et al.* 1991; Iwasa & Pomiankowski 1994, 1999).

There are many studies reporting phenotypic correlations between sexual ornament size and measures of condition (Andersson 1994; Johnstone 1995), but good experimental support for such relationships is lacking in the majority of cases (Cotton *et al.* 2004a; Chapter 2). Most studies have failed to employ appropriate controls, so it is often impossible to tell whether ornaments have evolved heightened condition dependence over and above that seen in other traits. The importance of body size scaling is usually neglected and unaccounted for, and the range or quality of controlled experimental conditions may be different from those experienced in nature. These shortcomings in experimental design severely limit our understanding of this most basic criterion of the handicap principle. In addition, the interpretation of these phenotypic data is greatly constrained in the absence of genetic information. There

have been strikingly few experimental investigations into the genetics of ornament condition dependence (reviewed in Cotton *et al.* 2004a; Chapter 2).

If exaggerated sexual ornaments signal male genetic quality, then we expect a genetic basis to condition dependence; males with the highest quality genotypes should have the largest sexual ornaments. However, this simplistic view needs to be extended to incorporate the interaction between environmental and genetic variation. As with other life history traits, condition is expected to possess a large environmentally determined variance component (Price & Schluter 1991; Houle 1992). By extension, highly condition-dependent sexual ornaments will also be strongly affected by the environment. The interactions between genetic and environmental factors are therefore crucial for our understanding of sexual traits, as they determine to what degree ornaments signal heritable benefits (Griffith *et al.* 1999).

Handicap theory predicts that males with high quality genotypes will produce larger ornaments (Grafen 1990; Iwasa *et al.* 1991; Iwasa & Pomiankowski 1994). This relationship should also hold for environmental variation if environmental stress has the same consequence for the differential cost of signalling (Iwasa & Pomiankowski 1999). The interaction between environmental and genetic quality variation can be further specified using the following formulation (A. Pomiankowski & Y. Iwasa *unpublished*),

$$q = \frac{ve}{1 + ve},$$

where individual quality (q) has both environmental (e) and genetic (v) components. This predicts that in a benign environment (large e), individuals will show relatively

little quality variation due to genotype, whereas in a stressful environment (small *e*), genetic differences will have an elevated effect on quality.

A number of consequences follow from this for variation in sexual ornament size (A. Pomiankowski & Y. Iwasa *unpublished*). First, the genetic variance in ornament size is predicted to increase with environmental stress. Second, the relative performance of genotypes, with respect to ornament size, is expected to be maintained across environments. Genotypes that do well in one environment should do well in others. However, as genotype has less consequence for ornament size in benign environments, the genetic correlation of ornament size across environments is likely to decrease as environments become more benign.

An additional expectation is that environmental stress will increase the variation of ornament size within genotypes as well as between genotypes (as explained above). This follows if random effects have greater consequences on the development of animals in stressful rather than unstressful environments. For example, a reduction in the quantity of food available to a group (or family) of animals will inevitably lead to some individuals receiving more food than others, for instance as a simple corollary of some individuals (siblings) being born or hatching earlier. In contrast, with abundant food, differences in the timing of egg hatch will not affect food availability. It may be a general relationship that stressful environments have more heterogeneous effects on individuals than benign environments, regardless of genotype. So we expect elevated ornament size variation within genotypes in more stressful environments.

As a result of heightened condition dependence, sexual ornaments are expected to be more sensitive to genetic and environmental factors than other non-sexual traits. Ornaments should show greater genetic and environmental variance,

greater increases in variance with stress and greater variance within genotypes than other non-sexually selected traits. High quality genotypes are predicted to be less sensitive to environmental stress and show less increase in variance with environmental heterogeneity.

The eye-stalks found in many species of stalk-eyed fly (Diptera: Diopsidae) are prime examples of sexually selected traits. The eyes of Diopsids are located on lateral extensions of the head capsule ("eye-stalks"), a character common to both sexes in all species (Wilkinson & Dodson 1997; Baker *et al.* 2001). The eyespan of males has become exaggerated in many species (Baker & Wilkinson 2001), the result of sexual selection through female choice (Wilkinson & Dodson 1997; Wilkinson *et al.* 1998), and inter-sexual competition (Panhuis & Wilkinson 1999).

Cyrtodiopsis dalmanni is highly sexually dimorphic for eyespan (Baker & Wilkinson 2001), with males having greatly exaggerated eye-stalks in comparison with those of females. Females exhibit mate preference for males with the largest absolute and relative eyespan (Wilkinson & Reillo 1994; Hingle *et al.* 2001). I have previously demonstrated that male eyespan in *C. dalmanni* shows heightened phenotypic condition dependence, both before and after controlling for body size, in comparison with male wing traits and female eyespan (Cotton *et al.* 2004b; Chapter 3) and unexaggerated male eyespan from species not under sexual selection (Cotton *et al.* 2004c; Chapter 4). Thus females can gain more information about male phenotypic condition by evaluation of male eyespan than by assessment of other traits or body size. I have also showed that phenotypic variance in eyespan increases as males become stressed, again, both before and after controlling for body size (Cotton *et al.* 2004b; Chapter 3).

A previous experiment using stalk-eyed flies provided evidence for a genetic basis of condition dependence in male eyespan (David *et al.* 2000). Using a full- and half-sib design, David *et al.* (2000) reared larvae from each family on three food quality types. Males from some families produced a large eyespan in each of the three environments, whilst the eyespan of males from others became progressively smaller as stress increased. This led to an increase in the genetic variance of absolute trait size with stress. David *et al.* (2000) found that the same patterns of genetic performance and variance persisted after they attempted to control for body size variation by using relative measures (trait size divided by body size). David *et al.* (2000; see also Maynard Smith & Harper 2003 p. 33) interpreted these findings as evidence for genetic variation in eyespan condition dependence.

Whilst it is clear that ornament ranks were maintained across environments and that genetic variance in absolute ornament size increased with stress, there were a number of potential problems with the study. First, the qualitative nature of the stresses used by David *et al.* (2000) resulted in a relative imbalance in the effects of each treatment on morphology. David *et al.* (2000) reared larvae from each family on corn, spinach and cotton wool to create environments of low, mild and high stress. Corn and spinach treatments produced adults of similar phenotypic size; larvae fed spinach eclosed as slightly smaller flies, but there was no significant difference between the groups in genetic variance. The main changes occurred in the worst environment, with flies reared on cotton wool becoming both smaller and more genetically variable than flies reared under more benign treatments. Thus much of the strength of David *et al.*'s (2000) findings was based on the (effectively single) qualitative comparison between corn/spinach environments with that of cotton wool.

It would be better to investigate environments that differed in a quantitative manner, in which the intermediate treatment represented a stress between the high and low stress regimes in its effect on morphology. This would allow useful comparisons to be made between high and moderate stressed flies and between moderate and low stressed flies. In addition, experimental control over the cotton wool treatment was less than that of other treatments because the cotton wool base of cages decayed unevenly and at different rates. So whilst all treatments were heterogeneous to some extent, the greater heterogeneity of the cotton wool may in itself have caused an amplification in the estimates of trait variation, both for genetic and phenotypic measures.

The second, and more serious, problem is that David *et al.* (2000) used relative measures to control for body size. Such procedures are only valid under the narrow condition of true isometry, where trait allometries are linear and pass exactly through the origin ($b = 1$ in the allometric equation $Y = aX^b$, where Y = trait size and X = body size). If the Y-intercept is not zero, then covariance with X remains and body size is not completely controlled for; negative allometric intercepts lead to relative Y-values (i.e. Y/X) exhibiting positive covariance with X, whilst positive Y-intercepts lead to negative correlations with X. In *C. dalmanni*, eyespan allometries have negative intercepts, so relative eyespan values tend to increase with body size; the Y-intercepts of female eyespan and wing traits are less negative and approach zero, respectively. Thus the disproportionate reductions in male relative eyespan reported by David *et al.* (2000) may be due in large part to the remnants of body size scaling that persist in relative measures. It therefore remains unclear whether there is a genetic basis to the body size-independent component of eyespan condition dependence found by Cotton *et al.* (2004b; Chapter 3). A related problem with the

David *et al.* (2000) study is that the per-family sample sizes were often too low (i.e. < 5) to permit a reasonable assessment of the within-line covariance between traits and body size. This was primarily a constraint arising from the experimental design, and it limits the use of more appropriate controls for body size (such as including body size as a covariate in General Linear Models; Packard & Boardman 1999; Darlington & Smulders 2001; García-Berthou 2001; Cotton *et al.* 2004a).

Finally, David *et al.* (2000) employed a full- and half-sib design to investigate the genetic basis of environmental condition dependence. Whilst the covariance between offspring from a common parent can be used to assess genetic variation in trait size and interactions between genotype and environment, the family units are genetically heterogeneous. In addition, individuals from some families shared only a sire, whilst individuals from others were full-sibs. This could cause problems in deducing genetic effects if the variation within genotypes is large and the sample size per sex per environment is low. A standard design used to reduce genetic variation within families is to create inbred lines, and this is the approach I follow here. The greater standardisation of genetic effects and the lower (within-line) genetic variance created by inbreeding also allows analysis of the change in variance across environments.

In this chapter I describe the genetics of condition-dependent ornament expression in *C. dalmanni*. I overcome the shortcomings of David *et al.* (2000) and extend the findings of my previous work (Cotton *et al.* 2004b; Chapter 3) by investigating the patterns of genetic variation in the body size-independent component of male eyespan expression. Rather than exactly replicating David *et al.*'s (2000) experimental design I employed a different stress that was quantitative rather than qualitative. By manipulating larval food availability I was able to draw from prior

knowledge about the relationship between levels of stress and morphology (Cotton *et al.* 2004b; Chapter 3), and create three treatments that produced a stepwise gradient of environmental stress. This allowed me to perform two meaningful contrasts; one between flies subjected no and moderate larval stress, and a second between moderately and highly stressed flies. I also perform appropriate control for body size by including body size as a covariate measure in General Linear Models as has been recommended recently (Packard & Boardman 1999; Darlington & Smulders 2001; García-Berthou 2001).

I overcame the design problem associated with full- and/or half-sib approaches by utilising a novel method that used experimental units with a high(er) level of within-group genetic similarity. I obtained information on different genotypes by creating a number of genetically distinct strains through repeated full-sib inbreeding. Flies from each inbred line were then reared under three larval stress regimes and the line reaction norms were determined for the male ornament (eyespan), the homologous female character and other non-sexual traits (wing length). Whilst there are potential drawbacks associated with this approach, such as the inability to partition genetic variance into additive and non-additive genetic components (see below), there are numerous benefits to it. For instance, sample sizes for each genotype (inbred line) tend to be higher than those using family-based designs, and the discrete and permanent nature of the inbred lines means that any findings can be extended in future generations (see Chapter 6). The performance of genotypes (inbred lines) was compared across treatments for each trait and changes in genetic variance were investigated to determine the nature of any genotype by environment interactions. The genetic basis of male eyespan expression was contrasted with that of other, non-sexual, traits and comparisons were performed on

both absolute trait size and body size-controlled measures. Patterns of standardised within-line variance were also investigated to test the hypotheses that ornaments have greater residual variance than other traits and that such differences in variance increase with stress.

5.3 MATERIALS & METHODS

5.3.1 Fly Rearing And The Production Of Inbred Lines

The laboratory-adapted population of *C. dalmanni* used in this experiment was derived from wild caught flies collected from Malaysia in 1993 by A. Pomiankowski. Flies have since been maintained in cage culture at 25°C on a 12 h:12 h light:dark cycle, and to minimise inbreeding the population size has been kept high (> 200 individuals).

To obtain distinct genotypes, a number of inbred lines were generated from the laboratory population (hereafter referred to as the base population (generation F_0)). F_0 flies were collected as virgins and kept in single sex cages until after maturity (> 4 weeks post eclosion; Baker *et al.* 2003). To initiate the lines, male-female F_0 pairs were chosen at random and allowed to mate freely. Each pair was housed in a separate container and eggs were collected twice weekly. Eggs were provided with excess food (Cotton *et al.* 2004b; Chapter 3). The resultant F_1 offspring were sexed and maintained in single sex groups.

At maturity, 5 full-sib F_1 male-female pairs were formed within each line and each F_1 pair was allowed to mate and produce the next (F_2) generation. Choice and pairing of F_1 flies was random within each sibship. One F_1 full-sib pair, which had produced

five mature F_2 offspring of each sex, was then chosen at random as parents of the F_2 generation of that line. The F_2 offspring of other F_1 pairs in that line were discarded. Occasionally, none of the F_1 pairs within a line produced 5 full-sib F_2 pairs. In such cases the F_1 pair used to propagate that line was chosen at random from those that produced ≥ 3 full-sib F_2 pairs.

This inbreeding cycle was repeated for a further generation to produce the F_3 generation, which was used to generate experimental flies for the condition-dependence assay (see below). In order to increase line productivity, three mature F_3 flies of each sex (rather than a single pair) were housed together and used as parents for the F_4 generation. The inbreeding coefficient (F) of flies used for measurements in this experiment was approximately 0.54 ($F_{F_4} = \Delta F + (1 - \Delta F)F_{F_3}$, where F_{F_n} = the inbreeding coefficient of generation n ($F_{F_3} = 0.50$ with full-sib inbreeding; Falconer & Mackay 1996 p. 90), ΔF = the incremental increase in inbreeding from F_3 to F_4 = $1/2N_e$ assuming that the effective F_3 population size contributing to the F_4 generation was equal to the actual population size (i.e. $N_e = 6$); Falconer & Mackay 1996 p. 60,66).

5.3.2 Manipulation Of Condition

Samples of 13 eggs were collected every other day from each group of six F_3 flies and reared on either 0.03 g or 0.015 g puréed corn per egg. Previous work had established that these food levels were stressful and produced flies of low or very low phenotypic condition respectively (Cotton *et al.* 2004b; Chapter 3). Flies in high phenotypic condition were produced by plating ≤ 13 eggs onto 1.56 g puréed corn.

Previous work had established that food supplied in excess of 0.12 g per egg was no stressful and produced flies of maximal size (Cotton *et al.* 2004b; Chapter 3).

5.3.3 Measurements

Emerging F_4 flies were collected, sexed and frozen. All individuals (n = 2554) were measured later to an accuracy of 0.01 mm using a monocular microscope and the image analysis program NIH Image (Version 1.55). Measurements were taken of eyespan, thorax and wing length (for details see Cotton *et al.* 2004b; Chapter 3). Wing measurements were taken from the left wing, unless this was damaged. Previous work has shown that larval stress has little or no effect on wing length fluctuating asymmetry (David *et al.* 1998; Bjorksten *et al.* 2000, 2001). All flies were measured 'blind' by a single person (SC).

5.3.4 Choice Of Data For Analysis And Summary Of Data Structure

Damaged traits were not measured. Flies with one or more damaged (and therefore un-measured) traits were excluded so that within-line, within-treatment estimates were based on the same flies for all three traits. This exclusion procedure removed less that 5% of the total. For within-treatment comparisons (of each sex separately) a line was declared 'present' if it had ≥ 5 flies. Lines with < 5 flies within a treatment were excluded from analyses containing that treatment. This ensured that a within-line, within-treatment variance component could be calculated with a reasonable degree of accuracy. For between-treatment comparisons (of each sex separately), only those lines that had ≥ 5 flies in each of the treatments were included

in analyses. Data from lines containing < 5 flies in each of the treatments were excluded from between-treatment analyses.

The dataset also contained a number of outliers. These were detected visually from distributions of data around the means of each line via inspection of the residual *vs.* predicted plot of the Model from a one-way ANOVA of LINE effects on each trait within each sex and each treatment. Outliers were obvious and were declared as such if they were ≥ 3.291 standard deviations away from the LINE mean. Assuming a Normal distribution model, 99.9% of items should be within $\overline{X}$ ± 3.291 SD. Outliers were also present when eyespan or wing traits were regressed on thorax length (pooled across all lines and all treatments, but for each sex separately). An estimated bivariate density ellipse containing 99.9% of items was fitted to the data. Items falling outside this area were considered outliers. This allometric outlier procedure was important as many stressed flies classified as males were actually females (based on their differing eyespan allometries); this was confirmed by subsequent re-inspection of fly genitalia. The 0.999 probability for inclusion of data was arbitrary, and conservative. A total of 25 flies were categorised as outliers and were removed from the dataset. This outlier exclusion procedure did not decrease the number of lines with ≥ 5 flies per treatment.

This cull of the data left 2375 flies from 23 inbred lines. The number of lines with ≥ 5 males in the > 0.12 g, 0.03 g and 0.015 g corn treatments were 22, 22 and 18 respectively. The number of lines with ≥ 5 females in the > 0.12 g, 0.03 g and 0.015 g corn treatments were 22, 21 and 18 respectively. 18 lines had ≥ 5 males in each of the three treatments, and 17 had ≥ 5 females in each of the three treatments.

5.3.5 Statistical Analysis

Absolute trait size

Genetic variation in trait size was detected as significant LINE effects in two-factor General Linear Models (GLM; LINE = random, FOOD = fixed). Eyespan and wing traits were analysed separately for each sex. The LINE × FOOD interactions from the GLMs were used to detect differences between lines in their response to food treatment.

Significant LINE × FOOD interactions could arise for two reasons. First, lines may exhibit crossover reaction norms, whereby a line that produces a large trait relative to other lines in one environment produces a smaller trait relative to other lines in another. This will produce negative correlations of trait size across treatments. This was investigated using two analytical methods. Pearson's product-moment correlation coefficients (r) were calculated for correlations between line *means* across food treatments, and Spearman's rank correlation coefficient (r_s) was used to assess correlations of the *ranks* of line means across treatments. Second, line reaction norms may diverge or converge, without crossing over (if correlations across treatments are positive) with consequent changes in the variance of trait size across environments. Divergence or convergence of reaction-norms was detected by changes in coefficients of variation (CVs) of line means across treatments. CVs were used to provide estimates of genetic variance that have been controlled for differences in trait size (e.g. Houle 1992; Pomiankowski & Møller 1995). Homogeneity of CVs across food treatments was tested using the Miller-Feltz method (Zar 1996, p. 206); within-trait, between-adjacent treatment contrasts were formalised using Z-tests compared to critical values from the t-distribution with infinite degrees of freedom (Zar 1996, p.

144). Comparisons of CVs were also performed within each treatment to identify differences between traits in their genetic variance.

I performed an explicit comparison between male eyespan and male wing length in a GLM with fixed factors of FOOD and TRAIT, and a random LINE effect. The significance of the three-way interaction (LINE × FOOD × TRAIT) indicates whether the genetic basis of condition-dependence differs between the two traits. An additional sexual *vs.* non-sexual trait comparison was performed between male eyespan and female eyespan, replacing the TRAIT effect with a SEX effect. A non-sexual comparison between male and female wing length was also undertaken as a control.

Body size-controlled trait size

Much of the response of trait size to stress results from body size scaling because flies are smaller when stressed as larvae (David *et al.* 1998, 2000; Cotton *et al.* 2004b; Chapter 3). The analysis of absolute trait size was therefore repeated using thorax length (THX) as an estimate of body size. THX was included as a covariate in General Linear Models (GLMs). Least Squares Means (LSMs) from within-treatment GLMs (with THX and LINE effects, and their interaction) were used to estimate mean line trait sizes after removing the effect of body size variation within each environment. Interactions between genotype and environment after the removal of body size scaling were visualised using across-treatment LSMs, derived as follows. Within-treatment LINE LSMs were derived for each trait (as above) and transformed to standardised normal deviates

$$y_{SND} = (y_{LSM:within} - \overline{y_{within}}) / SD_{y_{within}},$$

where $y_{LSM:within}$ is the LINE LSM trait size, and $\overline{y_{within}}$ is the mean LSM trait size (within each treatment). To ease comparisons across treatments, within-treatment LINE LSMs were transformed into across-treatment LINE LSMs using the following equation

$$y = y_{LSM:across}(y_{SND}CV_{y_{within}} + 1),$$

where $y_{LSM:across}$ = the LSM estimate of each treatment derived from an ANCOVA with FOOD and THX effects (plus the interaction), and $CV_{y_{within}}$ = the estimated coefficient of variation of LINE LSMs within each treatment ($CV_{y_{within}} = SD_{y_{within}} / \overline{y_{within}}$).

The LINE effects in a model containing THX, LINE (random) and FOOD (fixed) effects plus all significant or required interactions was used to assess genetic variation in trait size after controlling for body size, and differences between lines in the response of body size-controlled trait size to food treatment were detected by LINE × FOOD interactions. Again, eyespan and wing traits were analysed separately for each sex.

Correlations between within-treatment LINE LSMs were used to determine the nature of significant LINE × FOOD interactions across treatments. Again, correlations were estimated using Pearson's r and Spearman's r_s for LSMs and LSM ranks respectively. To assess convergence and divergence of reaction norms after removing the allometric covariance with body size I used estimates of allometric dispersion (AD; Cotton *et al.* 2004b; Chapter 3). AD is the coefficient of variation that Y (i.e. the trait of interest) would have if X (i.e. body size) were held constant ($AD = CV_Y(1 - r^2)^{0.5}$), where r is Pearson's product-moment correlation coefficient between Y and X. With respect to genetic variation, AD is the genetic variation in Y

after removing its genetic covariance with *X*. *r* was calculated as the within-treatment (genetic) correlation coefficient between the line mean trait sizes and line mean body sizes (thorax length). Homogeneity of ADs across food treatments was tested using the Miller-Feltz method as above, and within-trait between-adjacent treatment contrasts were performed using *Z*-tests. Comparisons of ADs were also undertaken within each treatment to identify differences between traits in their body size-independent genetic variance.

Explicit comparisons between body size-controlled male eyespan and male wing length were made (GLM with fixed factors of FOOD and TRAIT, a random LINE effect, and THX as a covariate), and the significance of the three-way interaction (LINE × FOOD × TRAIT) was used to reveal trait differences in the genetic basis of condition dependence. The four-way interaction between all variables was included if significant, otherwise all non- significant higher order interactions were omitted from the model. Additional comparisons were performed between male and female eyespan and between male and female wing length.

Trait variance

Measures of variance were estimated for each trait in each environment as within-line coefficients of variation (CVs). I used paired *t*-tests of within-line CVs across adjacent treatments to assess changes in variance with stress. This was performed for each trait separately. Trait variances were also compared within each treatment using similar paired *t*-tests. The relationship between trait size and variance was investigated in analyses of covariance with FOOD effects and line mean TRAIT SIZE as covariates. TRAIT SIZE and CV variables were Log_{10}-transformed to ensure that the residual variance of the model was homoscedastic. The direction of any TRAIT

SIZE effects was determined from the sign of the parameter estimate derived from the model.

To estimate the component of variance that was independent of body size I used the within-line allometric dispersion (AD). The correlation coefficient used to remove the covariance between CV and body size was calculated from the within-line, within-treatment correlation of trait size on body size (thorax length). Any non-significant correlations were assigned an *r*-value of zero. The same suite of tests was performed on within-line estimates of AD as with within-line CVs (except that within-line LSM estimates of trait size were used in ANCOVAs rather than within-line mean trait sizes).

5.4 RESULTS

5.4.1 Absolute Trait Size

There was significant variation between lines for eyespan and wing traits of each sex (LINE $F_{16\text{-}17,1003\text{-}1034} \geq 10.01$, all $P < 0.001$). Treatment had significant effects on both traits in each sex; flies became smaller as food quantity decreased (Figure 5.1; FOOD $F_{2,32\text{-}34} \geq 157.87$, all $P < 0.001$). Lines also differed significantly in their response to food stress (visible as non-parallel reaction norms in Figure 5.1). These differences occurred in both traits of each sex (LINE × FOOD $F_{32\text{-}34,1003\text{-}1034} \geq 4.32$, all $P < 0.001$).

There were significant positive correlations across lines between the two most stressful environments, 0.03 g and 0.015 g corn per egg (Table 5.1). This occurred in both traits of each sex. Lines that produced a large trait when moderately stressed also

produced a large trait when highly stressed. However, these line performances were not predicted by trait size in the least stressful (> 0.12 g per egg) treatment, as most correlations containing this group were not significantly different from zero. Only female wing showed significant positive correlations between the > 0.12 g and 0.03 g treatment groups. These patterns held when estimating correlations via line means or line ranks using Pearson's r or Spearman's r_s, respectively (Table 5.1).

Coefficients of variation (CVs) of line means are given in Figure 5.2. There was significant CV heterogeneity between treatments for both traits in each sex (male ES $\chi^2_2 = 33.12$, male wing $\chi^2_2 = 18.66$, both $P < 0.001$; female ES $\chi^2_2 = 11.89$, $P = 0.003$, female wing $\chi^2_2 = 11.27$, $P = 0.004$). Standardised genetic variation for trait size increased as flies became more stressed (Figure 5.2). The greatest increase of genetic variation with stress occurred in male eyespan (Figure 5.2, Table 5.2).

Explicit comparisons between traits revealed that the genetic basis of condition dependence was significantly different between male eyespan and male wing traits (LINE × FOOD × TRAIT $F_{34,2006} = 3.45$, $P < 0.001$). There were also significant differences between male eyespan and the homologous female trait (LINE × FOOD × SEX $F_{32,2068} = 2.28$, $P < 0.001$), and to a lesser extent for male and female wing traits (LINE × FOOD × SEX $F_{32,2002} = 1.53$, $P = 0.03$).

5.4.2 Body Size-controlled Trait Size

As with absolute trait size, there were significant differences between lines for both body size-controlled traits (LINE $F_{16\text{-}17,949\text{-}1015} \geq 3.32$, all $P < 0.001$), and significant differences in body size-adjusted trait size between treatments (Figure 5.3; FOOD $F_{2,32\text{-}34} \geq 10.35$, all $P < 0.001$). Eyespan LSMs decreased in both sexes as stress

increased, whereas the effect of FOOD on wing LSMs was small and inconsistent (Figure 5.3). There were also significant LINE × FOOD interactions for male eyespan (LINE × FOOD $F_{34,949} = 1.86$, $P = 0.002$), male wing (LINE × FOOD $F_{34,983} = 1.72$, $P = 0.007$) and female eyespan (LINE × FOOD $F_{32,1015} = 1.54$, $P = 0.03$), suggesting that there is genetic variation in the response of trait size to stress that acts independently of body size. These interactions are visualised as non-parallel reaction norms in Figure 5.3. Lines did not differ significantly for body size-controlled female wing length responses (LINE × FOOD $F_{32,983} = 1.38$, $P = 0.08$).

There were strong positive correlations of line performance between all three treatments (Table 5.3). The removal of body size revealed that lines differed consistently across environments in body size-controlled trait size; a line that performed well in one environment also performed well in others. This was true for both traits in each sex. These patterns held when estimating correlations via line means or line ranks using Pearson's r or Spearman's r_s, respectively (Table 5.3). So absolute trait size in benign environments is a random variable with respect to genotype (inbred line), but the non-allometric component of trait size remains consistently predictive of genotype across all levels of stress.

Estimates of allometric dispersion (AD) are shown in Figure 5.4. There was no evidence for significant heterogeneity of ADs for any trait (male eyespan $\chi^2_2 = 1.14$, $P = 0.57$; male wing $\chi^2_2 = 0.17$, $P = 0.91$; female eyespan $\chi^2_2 = 0.69$, $P = 0.71$; female wing $\chi^2_2 = 3.48$, $P = 0.18$; Figure 5.4), suggesting that body size-independent components of genetic variance were not affected by dietary treatment. However, there was a trend for male eyespan ADs to increase with stress whereas ADs of other traits tended to remain constant or decline (Figure 5.4). Within-treatment between-trait AD contrasts (Figure 5.4, Table 5.4) showed that there was significant

divergence between traits with stress. The eyespan AD of highly stressed males was significantly greater than that of female eyespan and marginally greater than that of male wing. These differences were not present in low or moderately stressed animals.

The differences between traits in their response of body size-independent genetic variance (AD) to stress were confirmed by explicit comparisons between traits in GLMs where thorax length was included as a covariate. The genetic basis of condition dependence differed between the sexes (LINE × FOOD × SEX $F_{32,1900} = 1.71$, $P = 0.016$) with a greater response in male eyespan (as judged by the greater AD of stressed males). Male eyespan and male wing length did not differ significantly (LINE × FOOD × TRAIT $F_{34,1898} = 1.39$, $P = 0.14$), and there was no sex difference in the genetic basis of wing condition dependence once the covariance with body size had been removed (LINE × FOOD × SEX $F_{32,1934} = 0.81$, $P = 0.76$).

5.4.3 Trait Variance

Absolute trait size variation

Within-line variation, as measured by coefficients of variation (CVs), increased significantly in both traits of each sex as flies became more stressed (Figure 5.5). Variance increased most markedly in male eyespan, and CVs were significantly greater in male eyespan than female eyespan and male wing length (in all treatments; Figure 5.5, Table 5.5). There was no consistent pattern in the differences between the variance of male and female wing traits (Figure 5.5, Table 5.5).

There were also significant associations between the line mean male eyespan and male eyespan CV. Lines with the largest ornaments tended to show the least amount of standardised variation in their ornaments (TRAIT SIZE $F_{1,58} = 8.40$, $P =$

0.005, TRAIT SIZE parameter estimate (± S.E.) = -1.45 ± 0.50). No such relationship was found for male wing length (male wing TRAIT SIZE $F_{1,58} = 3.52$, $P = 0.07$). However, CVs tended to decrease with trait size in females (eyespan TRAIT SIZE $F_{1,55} = 19.17$, $P < 0.001$, wing TRAIT SIZE $F_{1,55} = 17.37$, $P < 0.001$, TRAIT SIZE parameter estimates (± S.E.) = -3.27 ± 0.75 and –3.84 ± 0.92 respectively).

Body size-controlled trait variation

After removing the covariation with body size, the variance of male eyespan, as measured by within-line estimates of allometric dispersion (AD), still increased with stress (Figure 5.6). A similar pattern was also observed in females, but there were no changes in the variance of wing traits with stress in either sex (Figure 5.6). Again, male eyespan was significantly more variable than female eyespan and male wing length within each line (Figure 5.6, Table 5.6). However, there were no differences between male and female wing length ADs.

There was no relationship between male eyespan LSMs and male eyespan ADs (eyespan TRAIT SIZE $F_{1,57} = 0.16$, $P = 0.70$), or between the size and variance of any other body size-controlled trait (male wing TRAIT SIZE $F_{1,59} = 0.02$, $P = 0.89$, female eyespan TRAIT SIZE $F_{1,55} = 0.60$, $P = 0.44$, female wing TRAIT SIZE $F_{1,58} = 0.15$, $P = 0.70$).

5.5 DISCUSSION

One of the most controversial areas in sexual selection is the extent to which costly male ornaments signal heritable benefits. For the handicap hypothesis to work, exaggerated sexual traits are required to show two basic features: 1) heightened

condition-dependent expression, and 2) a genetic basis underlying that condition dependence. In general, good experimental evidence for the former is scarce, and almost completely lacking for the latter (reviewed in Cotton *et al.* 2004a; Chapter 2).

Previously, I showed that the size and variance of eyespan in male *C. dalmanni* exhibits heightened phenotypic condition-dependence compared to the homologous female trait and other non-sexual characters before and after controlling for body size (Cotton *et al.* 2004b; Chapter 3; see also Cotton *et al.* 2004c, Chapter 4). In this chapter I examined the genetics of sexual trait expression in a range of environments. I used inbreeding to generate distinct genotypes, and exploited the variation in trait size between inbred lines to estimate the genetic variance of male eyespan and a number of control traits (female eyespan and wing traits from each sex).

On the assumption of no dominance variance, the between-line variance approximates the additive genetic variance when $F \approx 0.50$ (Falconer & Mackay 1996, p. 264-265; Gordon 2003). However, the experimental design does not allow the partitioning of the genetic variance into additive and non-additive components. Significant dominance variance could lead to an overestimation of between-line components of (additive) genetic variance. In addition it is possible that the level of dominance variance may vary between the traits under consideration. Traits closely related to fitness (and hence subject to directional selection eroding their additive genetic variance) are predicted to have higher relative proportions of dominance variance than morphological traits that are typically under stabilising selection (reviewed in Roff 1997; see also Crnokrak & Roff 1995). Highly condition-dependent male ornaments might therefore be expected to exhibit greater levels of dominance variation than other morphological traits. Although David *et al.* (2000) did not

distinguish additive from non-additive genetic variation, they found that the dam component of variance was no greater than that of the sires. This suggests that non-additive variation does not explain a large fraction of the genetic variation in eyespan or wing size in *C dalmanni.*

This study confirms a number of findings reported previously by David *et al.* (2000), but also sheds new light on the genetic basis of ornament condition dependence in stalk-eyed flies. I found significant effects of genotype (inbred line) on male eyespan, and strong interactions between genotype and the environment. The nature of these interactions tended to preserve the underlying genetic signal; lines that produced a large absolute male eyespan when moderately stressed also produced a large ornament when highly stressed. These interactions mirror those found for absolute male eyespan by David *et al.* (2000). However, I found no relationship between line performance for absolute trait size of flies reared in the least stressful treatment and those reared in more stressful environments. This was not found by David *et al.* (2000), but is not surprising given the theory outlined in the Introduction. In an extremely benign environment, the cost of producing a large ornament is likely to be largely independent of individual genetic quality as differences between genotypes are masked, or very much reduced. It is only when flies become stressed that genetic variation in condition will tend to be amplified and revealed. This was the pattern that I found. Importantly, the maintenance of line performance in stressed treatments was also accompanied by large increases in the genetic variance of ornaments as the larval environment deteriorated. This finding, together with the maintenance of line ranks, confirms David *et al.*'s (2000) assertion that stress magnifies differences between genotypes for absolute ornament size. David *et al.* (2000) also found similar genetic patterns in female eyespan and wings, but they did

not compare the response of male eyespan with those of other traits. In this study I too observed that qualitatively similar genetic responses of sexual and non-sexual traits, but I explicitly tested, and found evidence in support of, the expectation that male eyespan has greater levels of genetic condition dependence than other traits.

There was also a genetic basis to the phenotypic patterns of condition dependence seen in the body size-independent components of male eyespan size and variation (Cotton *et al.* 2004b; Chapter 3). As with absolute eyespan, I found that genotypes responded differently to food treatment. Line performance for body size-adjusted male eyespan was strongly conserved across all treatments; a line that did well in one environment (i.e. produced a large eyespan for its body size) also did well in others. This finding differs from that of absolute trait size because absolute male eyespan in the benign environment was random with respect to line membership and only became a predictor of genotype under conditions of stress. However, the non-allometric component of male eyespan remained consistently predictive of genotype across all treatments, suggesting that it is a more sensitive or reliable metric of genotype than absolute trait size. After removing the genetic covariance with body size, the genetic variation of male eyespan also showed a trend of increasing with stress and became greater than that of other traits. Although, similar patterns were observed in some non-sexual traits, male eyespan tended to show the strongest genetic component of condition-dependence after controlling for body size. David *et al.* (2000) also claimed to have found such a result using relative measures (trait size/body size), but this interpretation can be criticised on the grounds that ratios do not adequately control for body size variation (see Introduction).

The genetic design employed here produced a higher degree of within-"family" genetic similarity than half- and full-sib approaches (e.g. David *et al.* 2000;

Kotiaho *et al.* 2001). This allowed me to examine the predicted effects of stress on within-genotype variance. Investigation of within-line variance components revealed that male eyespan was more variable than other traits, and that such differences increased with stress more in the male ornament than other traits. These patterns persisted even after controlling for body size. In addition, there was a negative relationship between the absolute mean size of a line's ornament and the standardised within-line variance, suggesting that, presumably higher quality, genotypes that produced the largest male eyespan were also better buffered against micro-environmental variation and developmental pressures. However, these correlations were also present in other traits, and did not remain when the covariance with body size was removed.

What are the evolutionary implications of these findings? Most importantly, they provide strong evidence for a "good genes" mechanism of sexual selection. The greater increases in the standardised genetic variance of eyespan in male *C. dalmanni* compared to other traits provide good indirect evidence that ornaments are subject to higher differential costs, where the cost is mediated by both genotype and the environment. This leads to the conclusion that male eyespan demonstrates heightened genetic and environmental condition dependence (this chapter; Cotton *et al.* 2004b; Chapter 3). All of these patterns are predicted by the condition-dependent handicap hypothesis (Andersson 1986; Pomiankowski 1987, 1988; Grafen 1990; Iwasa *et al.* 1991; Iwasa & Pomiankowski 1994, 1999). A female who preferentially mates with large eyespan males will therefore produce offspring in higher genetic condition than a female who mates at random. This advantage will be greatest in the poorest environments as stress enhances differences between genotypes with respect to

ornament expression. However, future investigation is required to link the genetic condition signalled by male eyespan with offspring fitness.

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Table 5.1. Correlations of line mean trait size across food treatments for eyespan (ES) and wing length (wing) traits. Pearson's *r* correlation coefficients are below the diagonal, Spearman's r_s correlation coefficients are above the diagonal. Asterisks denote the significance of correlation: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Trait	Treatment	0.015 g	0.03 g	> 0.12 g
Male ES	0.015 g		0.63**	-0.20
	0.03 g	0.54*		-0.05
	> 0.12 g	-0.19	0.07	
Male wing	0.015 g		0.70**	-0.07
	0.03 g	0.62**		-0.03
	> 0.12 g	0.08	0.16	
Female ES	0.015 g		0.84***	-0.05
	0.03 g	0.77***		0.26
	> 0.12 g	-0.18	0.30	
Female wing	0.015 g		0.80***	0.33
	0.03 g	0.84***		0.62**
	> 0.12 g	0.40	0.64**	

Table 5.2. *Z*-values for between-trait, within-treatment contrasts of coefficients of variation (CVs) in eyespan (ES) and wing traits.

Treatment	male ES vs. male wing	male ES vs. female ES	male wing vs. female wing
> 0.12 g	1.60	-1.34	-1.92
	($P = 0.11$)	($P = 0.18$)	($P = 0.06$)
0.03 g	3.74	3.71	1.07
	($P < 0.001$)	($P < 0.001$)	($P = 0.29$)
0.015 g	4.28	3.23	-0.13
	($P < 0.001$)	($P = 0.001$)	($P = 0.90$)

Table 5.3. Correlations of line Least Squares Mean trait size across food treatments for eyespan (ES) and wing length (wing) traits. Pearson's r correlation coefficients are below the diagonal, Spearman's r_s correlation coefficients are above the diagonal. Asterisks denote the significance of correlation: $^{**}P < 0.01$, $^{***}P < 0.001$.

Trait	Treatment	0.015 g	0.03 g	> 0.12 g
Male ES	0.015 g		0.54**	0.79***
	0.03 g	0.64**		0.69**
	> 0.12 g	0.83***	0.77***	
Male wing	0.015 g		0.73***	0.87***
	0.03 g	0.78***		0.87***
	> 0.12 g	0.88***	0.84***	
Female ES	0.015 g		0.71***	0.61**
	0.03 g	0.75***		0.86***
	> 0.12 g	0.62**	0.86***	
Female wing	0.015 g		0.80***	0.88***
	0.03 g	0.78***		0.92***
	> 0.12 g	0.81***	0.88***	

Table 5.4. *Z*-values for between-trait, within-treatment contrasts of allometric dispersion (AD) in eyespan (ES) and wing traits.

Treatment	male ES vs. male wing	male ES vs. female ES	male wing vs. female wing
> 0.12 g	0.53	0.58	-0.06
	($P = 0.59$)	($P = 0.56$)	($P = 0.95$)
0.03 g	1.02	0.90	2.64
	($P = 0.31$)	($P = 0.37$)	($P = 0.008$)
0.015 g	1.79	2.39	2.06
	($P = 0.07$)	($P = 0.02$)	($P = 0.04$)

Table 5.5. Paired *t*-tests of within-line coefficients of variation (CVs) for within-treatment, between-trait contrasts.

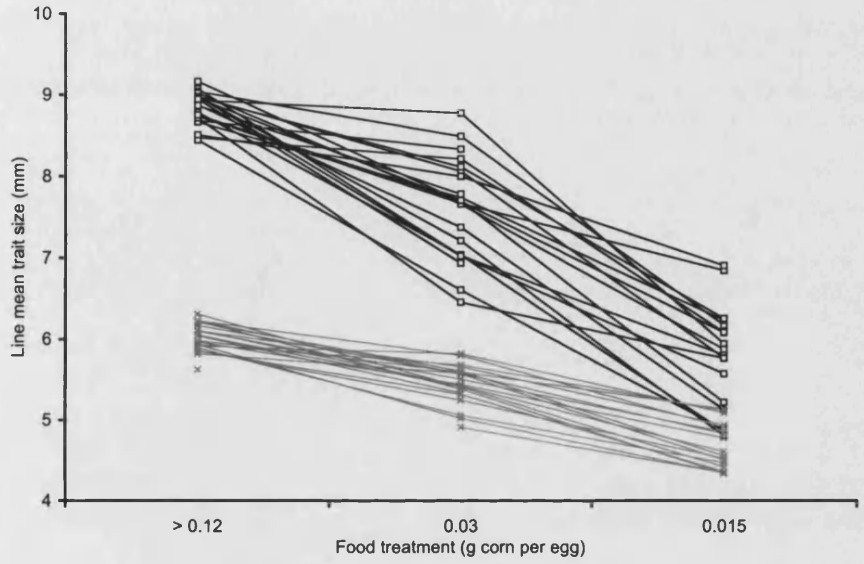
Treatment	male ES vs. male wing	male ES vs. female ES	male wing vs. female wing
> 0.12 g	$t_{21} = 9.62$	$t_{20} = 4.27$	$t_{20} = -0.55$
	$P < 0.001$	$P < 0.001$	$P = 0.59$
0.03 g	$t_{21} = 13.57$	$t_{19} = 6.50$	$t_{19} = 2.18$
	$P < 0.001$	$P < 0.001$	$P = 0.042$
0.015 g	$t_{17} = 9.55$	$t_{16} = 4.61$	$t_{16} = 0.98$
	$P < 0.001$	$P < 0.001$	$P = 0.34$

Table 5.6. Paired *t*-tests of within-line estimates of allometric dispersion (AD) for within-treatment, between-trait contrasts.

Treatment	male ES vs. male wing	male ES vs. female ES	male wing vs. female wing
> 0.12 g	$t_{20} = 3.81$	$t_{20} = 4.50$	$t_{20} = -0.02$
	$P < 0.001$	$P < 0.001$	$P = 0.99$
0.03 g	$t_{20} = 8.00$	$t_{19} = 5.30$	$t_{19} = 0.24$
	$P < 0.001$	$P < 0.001$	$P = 0.81$
0.015 g	$t_{17} = 6.67$	$t_{16} = 2.28$	$t_{16} = 0.04$
	$P < 0.001$	$P = 0.038$	$P = 0.97$

Figure 5.1. Reaction norms of a) absolute eyespan and b) wing traits. Each line represents the mean trait size of flies from an inbred line raised in the three food treatments. Male reaction norms are displayed in black whilst those of females are in grey.

a



b

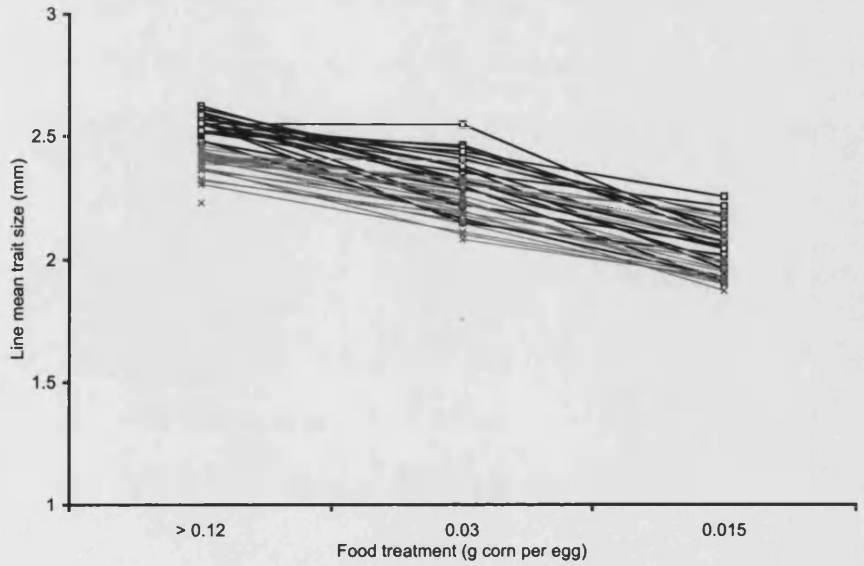


Figure 5.2. Coefficients of variation (CV ± SE) for line mean eyespan (ES) and wing length (Wing). Asterisks indicate significant differences between CVs in adjacent treatments: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

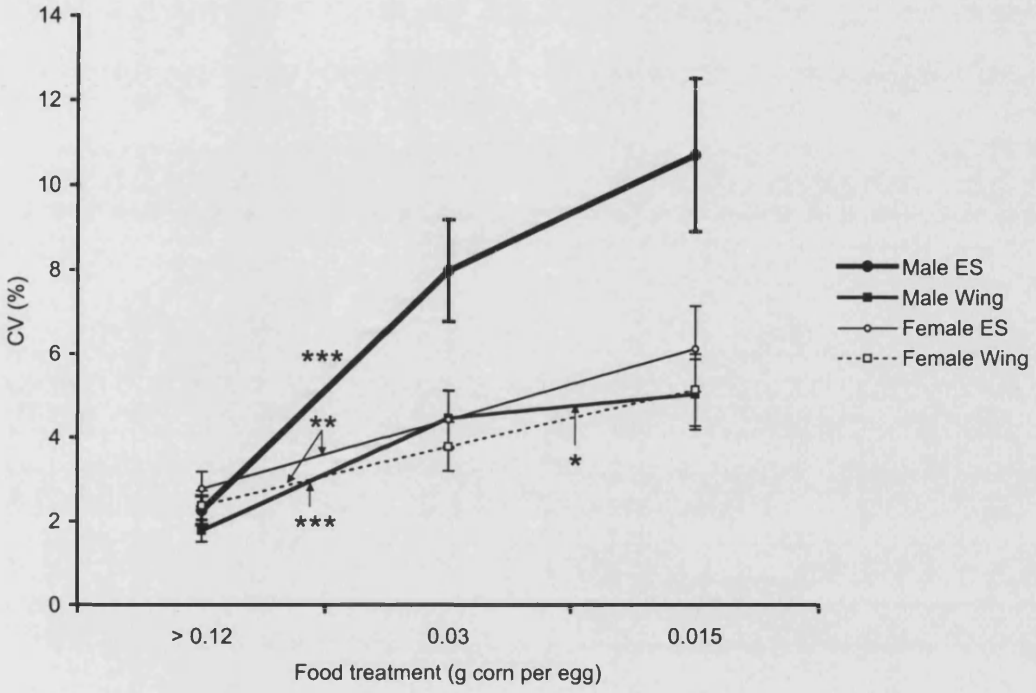
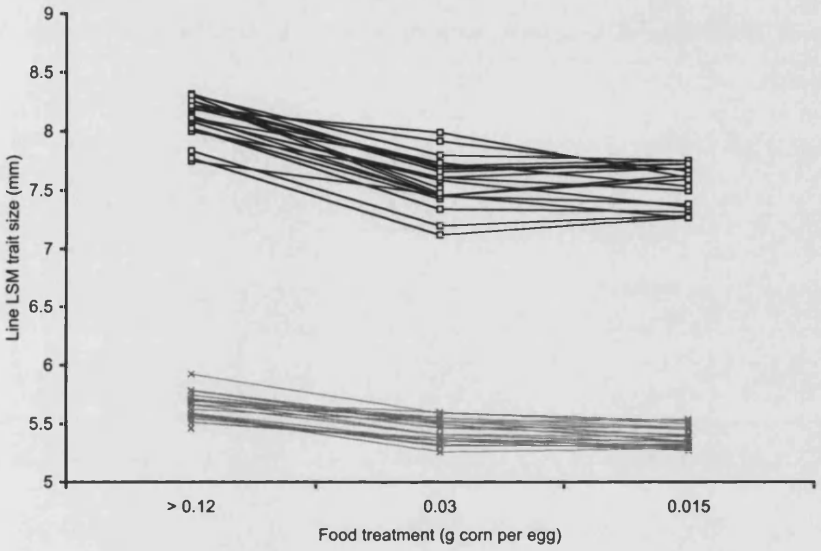


Figure 5.3. Reaction norms of a) eyespan and b) wing traits after controlling for body size variation. Each line represents the Least Squares Mean (LSM) estimate of trait size of flies from an inbred line raised in the three food treatments (see Materials & Methods § for derivation). Male reaction norms are displayed in black whilst those of females are grey.

a



b

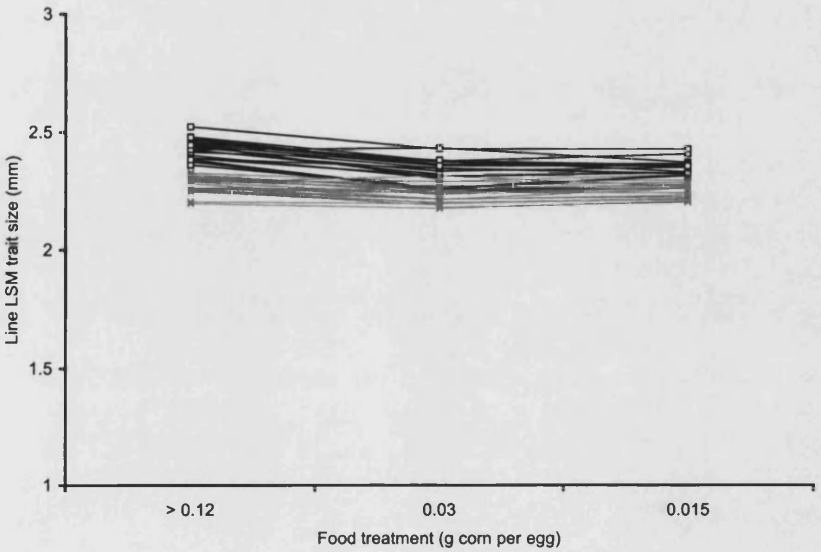


Figure 5.4. Allometric dispersion (AD ± SE) of eyespan (ES) and wing length (Wing). Asterisks indicate significant differences between ADs in adjacent treatments: $*P < 0.05$.

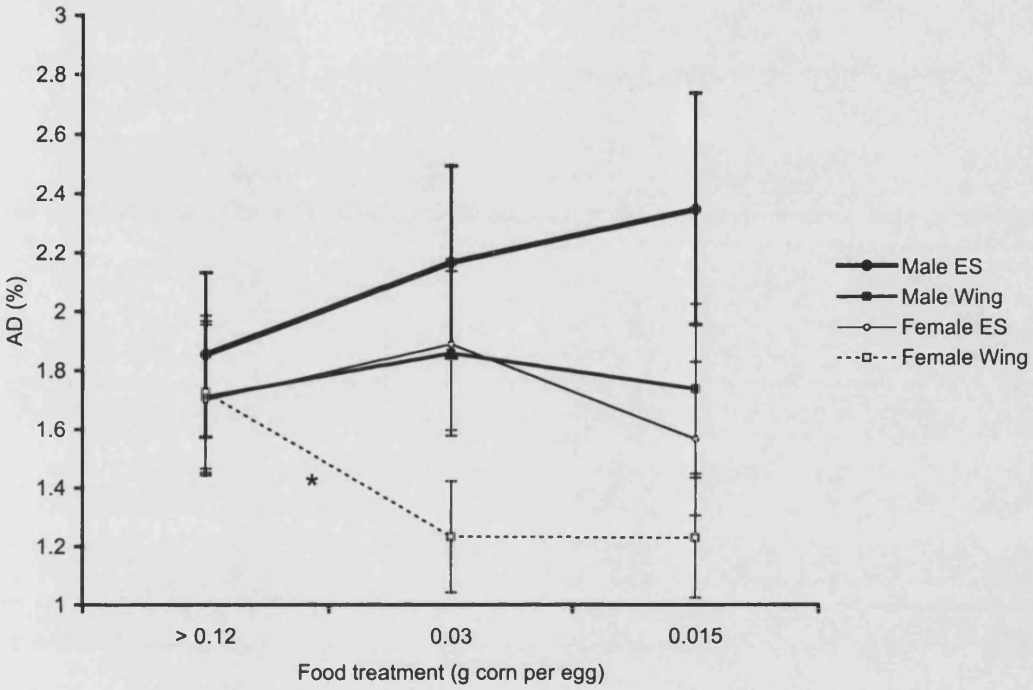


Figure 5.5. Mean within-line coefficients of residual variation (CVs) of eyespan (ES) and wing length (Wing). Asterisks denote significance of paired *t*-tests of within-line CVs between adjacent treatments. *** $P < 0.001$, ** $P < 0.01$.

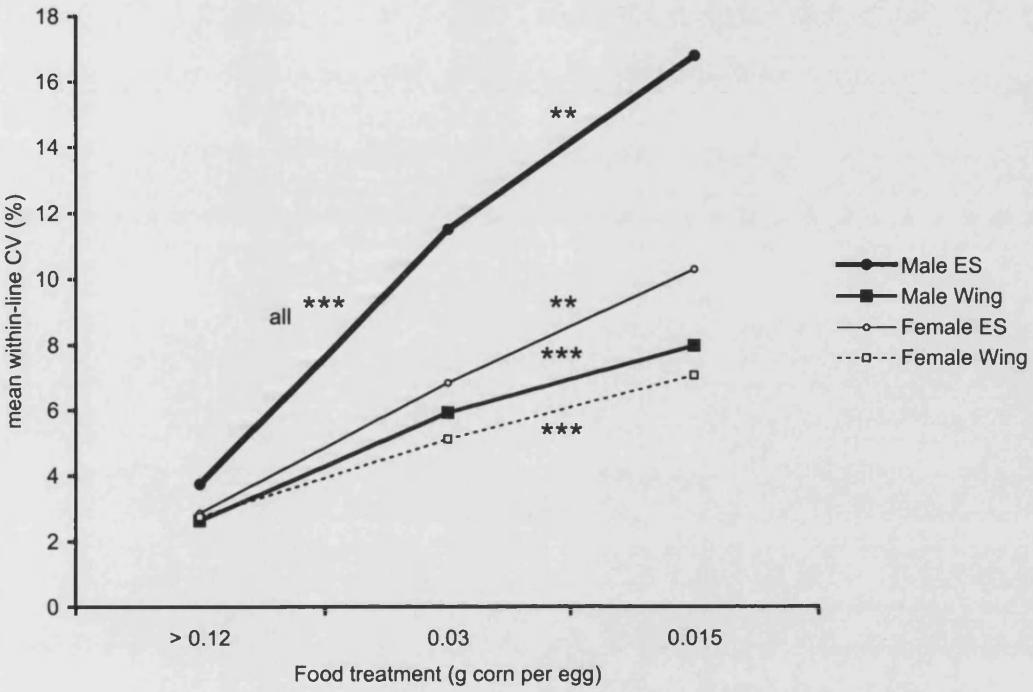
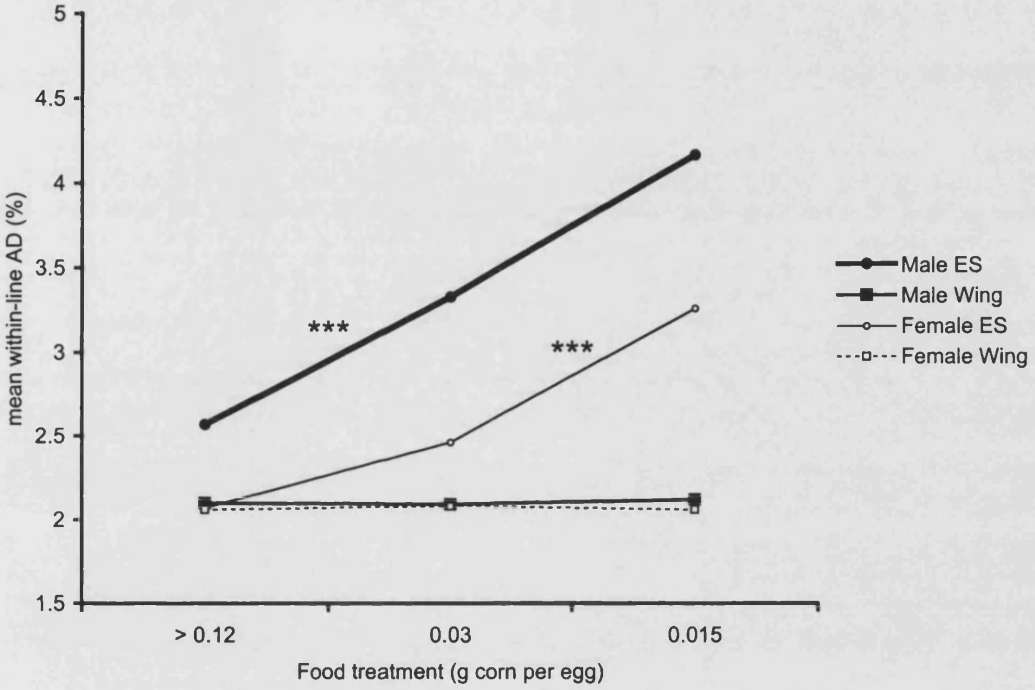


Figure 5.6. Mean within-line estimates of allometric dispersion (ADs) of eyespan (ES) and wing length (Wing). Asterisks denote significance of paired *t*-tests of within-line ADs between adjacent treatments. *** $P < 0.001$.



6

Does Male Eyespan Covary Genetically With Components Of Fitness In The Stalk-Eyed Fly, *Cyrtodiopsis dalmanni* (Diptera: Diopsidae)?

6.1 ABSTRACT:

"Good genes" models of sexual selection posit that male sexual ornaments signal male genetic condition. Females who choose to mate with well-adorned males are therefore expected to accrue such beneficial genetic qualities for their offspring. This leads to the prediction that male ornament size indicates high breeding values of one or more components of fitness. I tested this prediction in the stalk-eyed fly *Cyrtodiopsis dalmanni* (Diptera: Diopsidae). Male eyespan in this species has become highly exaggerated as a result of female mate preferences. It demonstrates heightened sensitivity to environmental and genetic condition and so has strong potential for signalling "good genes". Inbreeding was used to create distinct genotypes, and between-line (genetic) correlations of male eyespan expression and four components of adult fitness (male fertility, female fecundity, and male and female longevity) were examined. I found that variation in male eyespan did not covary with female fecundity or either component of male fitness. However, after adjusting for body size, variation in male eyespan was negatively correlated with estimates of female longevity. This was unexpected, and possible explanations for such a correlation are discussed.

6.2 INTRODUCTION

In many species, females have mate preferences for males with elaborate sexual traits (Andersson 1994). Handicap models of sexual selection propose that costly mate choice evolves for ornaments that signal male quality (i.e. for traits that exhibit elevated condition-dependent expression; Andersson 1986; Pomiankowski 1987a, 1988; Grafen 1990; Price *et al.* 1993; Iwasa *et al.* 1991; Iwasa & Pomiankowski 1994, 1999). The handicap principle works if the costs of female choice are more than offset by increases in offspring fitness derived from the greater quality of well-ornamented males.

A fitness advantage can be accrued through either non-heritable benefits that directly improve female reproductive success (Price *et al.* 1993; Iwasa & Pomiankowski 1999), or by an indirect genetic component. The latter, "good genes", hypothesis has received a wealth of theoretical (e.g. Pomiankowski 1987a, 1988; Grafen 1990; Iwasa *et al.* 1991; Iwasa & Pomiankowski 1994; Houle & Kondrashov 2002; Kokko *et al.* 2002) and empirical (reviewed in Andersson 1994; Møller & Alatalo 1999; Jennions *et al.* 2001) attention.

The nature of any "good genes" signalled by male sexual traits is difficult to discern. Kokko (2001; *et al.* 2002) has advocated an all-encompassing view of the "good genes" label by using it as an umbrella term for any, or all, heritable aspects of offspring fitness. This includes the expected mating success of male offspring, a component of fitness traditionally assigned to Fisher's runaway model of sexual selection (Fisher 1930; Lande 1981; Kirkpatrick 1982; Pomiankowski *et al.* 1991). Under this definition, any ornament with a genetic basis is itself a signal of "good genes" for male attractiveness.

Of greater interest is the possibility that ornaments reflect naturally selected components of fitness as well as sexual attractiveness. This was first proposed by Fisher (1915, 1930) as the initial catalyst for his runaway process, and has come to dominate discussions of "good genes" sexual selection (e.g. Møller & Alatalo 1999; Jennions *et al.* 2001). Models of the handicap principle have employed a single "viability" or "genetic condition" trait as a proxy for "good genes" (e.g. Pomiankowski 1987a,b, 1988; Grafen 1990; Iwasa *et al.* 1991; Iwasa & Pomiankowski 1994). Following Maynard Smith (1987 p. 12), I use the term "viability" (or "genetic condition") to mean components of fitness other than male attractiveness, such as longevity and fertility/fecundity. However, it is unrealistic to assume that such a simplification extends to nature; sexual traits will most likely signal some major component(s) of fitness rather than viability in its entirety. Theory does not predict which components of fitness these will be, especially in light of the complex relationships between life-history traits (Stearns 1992). Ideally, investigation of "good genes" sexual selection requires knowledge of the genetic covariance between all major fitness components and male ornament expression. This includes the genetic relationships of fitness between sires and daughters, as viability benefits can equally arise through elevated female longevity and/or reproductive success. Consequently a rigorous approach is to assess a suite of components of viability.

The basic assumption, that ornaments exhibit heightened condition dependence, remains, in many cases, untested, and the differential importance of allometric and non-allometric components of ornament size has been overlooked (Cotton *et al.* 2004a; Chapter 2). In addition, only a handful of studies have experimentally investigated the genetic basis of condition-dependent ornament expression (e.g. David *et al.* 2000; Chapter 5), so it is unclear to what extent

ornaments signal heritable properties. We expect high quality male genotypes to express the largest sexual ornaments, and that this relationship between ornament size and condition will persist when genotypes are reared along a gradient of environmental stress. Thus theory predicts that "good genes" will be signalled not only by sexual ornament size, but also by the reaction norms of ornament size.

In this chapter I examine the "good genes" signalling potential of male ornaments in the stalk-eyed fly, *Cyrtodiopsis dalmanni* (Diptera: Diopsidae). *C. dalmanni*, like other Diopsids, exhibits hypercephaly, the lateral elongation of the head into stalks on which the eyes are located. As a result of sexual selection, males have a greatly exaggerated eyespan (Baker & Wilkinson 2001; Wilkinson & Dodson 1997). *C. dalmanni* inhabits riparian regions of South-East Asia and forms nocturnal mating aggregations on root hairs overhanging the banks of streams (Burkhardt & de la Motte 1985; Wilkinson & Dodson 1997). Males fight for control of these roosting sites (contests are usually won by males with the largest eyespan; Wilkinson & Dodson 1997; Panhuis & Wilkinson 1999) and females prefer to roost and mate with males possessing the largest absolute and largest relative eyespans (Wilkinson & Reillo 1994; Hingle *et al.* 2001a).

Previously, I demonstrated that male eyespan shows heightened condition dependence relative to female eyespan and other, non-sexual, traits (Cotton *et al.* 2004b; Chapter 3; see also Cotton *et al.* 2004c, Chapter 4). In addition, there was a strong genetic component underlying the environmental condition dependence of the male ornament. Using inbreeding to create distinct genotypes, I found that an inbred line that produced a large eyespan in one environment tended to do so in other environments as well, and that these differences between genotypes were magnified by environmental stress (Chapter 5). These patterns held both before and after

controlling for body size variation. Thus male eyespan is a more sensitive indicator of genotype than other traits and body size.

Here I extend the morphological findings from these inbred lines by investigating between-line (genetic) correlations of male eyespan with components of fitness. I tested the hypothesis that male eyespan is positively correlated with viability, and that females obtain genetic benefits for their offspring by mating with well-ornamented males. Theory does not predict which components of fitness male ornaments will signal, so I decided to investigate a suite of potentially important aspects of adult viability: male fertility, female fecundity and male and female longevity. A draw back of this approach is that any patterns seen in one component may be counterbalanced by contrasting patterns in other, unmeasured, components. This possibility is discussed below. The discrete nature of the inbred lines meant that I was able to obtain correlative information over more than one generation, allowing me to assess more components of fitness and with greater sample sizes than traditional genetic designs typically constrained by limits on family size. Examination of fitness components was performed under a single regime of moderate adult stress in an attempt to increase the variance between genotypes. Positive correlations were sought between components of viability and measures of male eyespan from each of the three larval environments used in Chapter 5, both before and after controlling for body size. This enabled me to assess the relationships between fitness components and the reaction norms of allometric and body size-independent components of male eyespan. *A priori* I have knowledge that male eyespan is a more sensitive indicator of genotype than other traits or body size (Chapter 5), so I also examined correlations between components of viability and non-sexual traits in order to test the premise that

the signalling function of male ornaments has evolved (Cotton *et al.* 2004a; Chapter 2).

6.3 MATERIALS AND METHODS

6.3.1 Fly Rearing And The Production Of Inbred Lines

The inbred lines used in this experiment were the same as those used in Chapter 5, and were derived from a laboratory-adapted population founded from wild-caught Malaysian *C. dalmanni* collected in 1993 by A. Pomiankowski. Since domestication, all flies have been maintained at 25°C on a 12 h:12 h light:dark photoperiod with 15 minute "dawn" and "dusk" periods of reduced light.

To obtain discrete genotypes, a number of inbred lines were formed from the laboratory base population by repeated full-sib mating over three generations (see § 5.3.1 in Chapter 5 for more details). Three mature F_3 flies of each sex (rather than a single pair as in the previous generations) were housed together and used as parents to produce flies for the morphology condition-dependence assay (see below). Before being culled for measurement, a randomly selected sample of three F_4 males and three F_4 females from each line of the morphology condition-dependence assay were used to generate the next (F_5) generation of the inbred lines. F_5 offspring from each line were used to assess male fertility and female fecundity. A randomly chosen sample of three F_5 males and three F_5 females from each line that were not used in the fertility/fecundity assays were set-aside as parents for the F_6 generation. F_6 offspring were used to estimate the male and female longevity for each line. So over three consecutive generations the morphological (F_4), reproductive (F_5), and longevity (F_6)

characteristics of each line were assessed. It was assumed that the genetic changes that took place over this period were small enough to allow these properties to be taken as characteristic of each line.

6.3.2 Morphological Condition Dependence

Randomly chosen samples of 13 eggs were collected every second day from each group of six F_3 flies and reared on either 0.03 g or 0.015 g puréed corn per egg, producing flies of low and very low phenotypic condition respectively (Cotton *et al.* 2004b; Chapter 3). Flies in high phenotypic condition were produced by plating ≤ 13 eggs onto 1.56 g puréed corn (i.e. > 0.12 g per egg, food supplied in excess; Cotton *et al.* 2004b; Chapter 3). Emerging F_4 flies were collected, sexed and frozen. A small sample of each line was reserved for propagating the next generation; these were collected at a later date. Morphology of holometabolous insects is fixed at eclosion so data for these progenitor flies was not biased by their late cull. All flies were measured 'blind' by a single person (SC) for eyespan, wing length and thorax length, an estimate of body size (see Chapter 5 for details).

6.3.3 Male Fertility

Fertility was estimated as the percentage of fertile eggs laid by an excess of females over a 12-day period after being exposed to a male for 18 hours (inclusive of one "dawn" period and one "dusk" period). F_5 males were raised under low larval density and supplied with puréed corn *ad libitum* for at least 30 days post eclosion to ensure that sexual maturity was reached (Baker *et al.* 2003). Flies were kept as

virgins. In order to inflict physiological stress, males from each line were maintained in groups of three on an *ad libitum* food source of 50% puréed corn: 50% methylcellulose (by volume) for 8 days prior to the assay. Methylcellulose is an indigestible bulking agent (Boots & Begon 1994; Knell *et al.* 1999), so food quality was reduced without limiting its availability. Males were housed in circular 400 ml plastic containers (95 mm high × 75 mm diameter) with a roosting string and a moist tissue paper base. During this time, males were also housed with three mature base stock females to allow them to mate. At 17:00 h on the eighth day, females were removed and males were transferred individually into similar containers with 7 randomly chosen mature virgin base stock females. A 7:1 sex ratio was chosen as pilot work indicated that it enhanced discrimination between males with respect to fertility and eliminated egg production as a limiting factor for fertility (S. Cotton *unpublished data*). Each group of 8 flies were allowed to mate at will until 11:00 h the following day, whereupon males were removed.

Eggs were collected from each group of 7 females over three consecutive 3-day periods following mating. The tissue paper base was removed from each container and stored at 25°C on a damp cotton pad in a Petri dish. Fertilised *C. dalmanni* eggs typically hatch 2-3 days after being laid, leaving only the outer shell of the chorion; unhatched eggs appear full with the embryo still inside the chorion (Baker *et al.* 2001). Hatching success of eggs was determined visually under a dissecting microscope after 5 days of incubation. Some unhatched eggs showed segmental striations and other signs of development and were classified as fertile. Females were fed 100% corn throughout the experiment.

6.3.4 Female Fecundity

Female fecundity was estimated as the total number of eggs laid over a 9-day period soon after sexual maturity. F_5 females were reared under low larval density and fed 100% puréed corn *ad libitum* for > 30 days post eclosion in the absence of males. This ensured that all females were sexually mature virgins (Baker *et al.* 2001). Groups of three females from each line were then maintained on a diet of 50:50 puréed corn:methylcellulose (by volume) to inflict physiological stress. This dietary stress was chosen as previous work and pilot studies indicated that it would depress female fecundity (Hingle *et al.* 2001b; S. Cotton *unpublished data*). Food was supplied in excess to minimise competition. Three randomly selected base stock males were also added to each of the containers at the start of the food treatment to stimulate egg production (Baker *et al.* 2001). Flies were housed in circular plastic containers (400 ml; 95 mm high × 75 mm diameter) with a roosting string and a moist tissue paper base.

After 8 days of food stress, females from each line were housed singularly in similar containers. Eggs were collected from each female over four consecutive 3-day periods and fecundity was estimated from the number of eggs deposited on the moist tissue paper base and the food (tray). Eggs laid elsewhere (e.g. the sides of the container, ventilation gauze) were few and were not included in the fecundity count. All females were fed their prescribed food source throughout the experiment.

6.3.5 Male And Female Longevity

Longevity was estimated as survival duration after eclosion. The F_6 generation used for assaying longevity were reared as larvae under low density (food supplied in

excess). Eclosing flies were collected every three days and housed individually in circular 400 ml plastic containers containing a roosting string and a damp cotton wool base. Animals were maintained within these containers and fed twice weekly on a low quality diet of 50% puréed corn: 50% methylcellulose for the duration of the experiment. Deaths of individual flies were recorded during feeding (every 3-4 days).

6.3.6 Statistical Analysis

Male eyespan condition dependence

The performance of a genotype (inbred line (LINE)) with respect to ornament expression was determined for each line by calculating the mean eyespan for each line when reared in each of the three food treatments (see Chapter 5 for an extensive discussion of the analysis of the genetic basis of condition dependence in *C. dalmanni*). Genetic variation in male eyespan was detected as significant LINE effects in a one-way ANOVA of trait size (within each treatment).

A significant proportion of the morphological response to stress results from differences in body size between environments and genotypes (David *et al.* 1998, 2000; Cotton *et al.* 2004b; Chapters 3, 5). The measurement of thorax length (THX) was therefore taken as a general indicator of male body size and included as a covariate in a General Linear Model (GLM) with factors LINE, THX and their interaction. Least squares means (LSMs) from within-treatment GLMs were used to estimate the relative mean line trait sizes after removing the effects of body size variation within each environment. Differences between male eyespan LSMs were detected through the significance of LINE effects in the within-treatment GLMs.

To ensure that reasonable estimates of line mean and LSM eyespan could be calculated only those lines with ≥ 5 males per treatment were included.

Male fertility

To estimate the fertility of males from each line, the total number of eggs fertilised by each male within each group of 7 females was summed across the three consecutive 3-day periods following mating. The total number of eggs produced by each group of 7 females was also summed over the same 9-day period. Previous studies (e.g. Baker *et al.* 2001; see also review in Arnqvist & Nilsson 2000) have estimated fertility as the percentage of eggs fertilised. Such practices can incur problems similar to those encountered when trying to control for body size using ratios (Packard & Boardman 1999; Cotton *et al.* 2004a; Chapter 2). The estimate of fertility will be biased if the ratio covaries with the total number of eggs, for example, if the proportion fertilised is higher when females lay fewer eggs. Here the percentage of fertile eggs and the number of eggs fertilised was strongly correlated ($r = 0.68$ $P < 0.001$), but there was no significant association between the percentage of fertile eggs and the total number of eggs ($r = 0.003$ $P = 0.97$). % fertility was therefore used as an unbiased estimate of a male's reproductive ability. To obtain realistic estimates of line fertility, only those lines with data from ≥ 5 males were included.

The % fertility variable was normally distributed so parametric analyses were used. For logistical reasons and due to the low productivity of many lines it was necessary to conduct the male fertility assay in three "blocks". A BLOCK variable was introduced to control for any differences between groups that were set up at different times. % fertility was analysed in a GLM with factors LINE and BLOCK. The LINE × BLOCK interaction was omitted as not all lines were represented in every experimental

block, and the significance of LINE and BLOCK terms was calculated using the ERROR mean square as the denominator in *F*-ratios. LSMs derived from the GLM were used as estimates of line % fertility after controlling for the significant BLOCK effect (BLOCK $F_{2,88} = 3.47$ $P = 0.036$).

Female fecundity

Fecundity was estimated for each female by summing the number of eggs recorded over the four 3-day collection periods. The female fecundity assay was performed in three separate blocks because of constraints similar to those affecting the estimates of male fertility. Data from blocks 1 and 2 were non-normally distributed, with many females having a fecundity of zero. No standard transformations could normalise the data. Non-parametric Kruskal-Wallis tests were therefore used to identify block effects. There were significant fecundity differences across blocks ($\chi^2_2 = 52.16$ $P < 0.001$); block 3 was significantly different from the two other blocks (*vs.* block 1 $\chi^2_1 = 47.31$, *vs.* block 2 $\chi^2_1 = 36.28$, both $P < 0.001$), but there were no differences between blocks 1 and 2 ($\chi^2_1 = 0.30$ $P = 0.58$). Data from blocks 1 and 2 were therefore pooled together, and block 3 was analysed separately.

Block 1 and 2. Fecundities were non-normal and could not be transformed to meet the assumptions of parametric tests. Non-parametric Kruskal-Wallis tests were used to look for significant differences between lines in female fecundity. Only those lines that had ≥ 5 females were included in the analysis (and subsequent correlations). The median number of eggs laid by females from each line was used as an estimate of line fecundity.

Block 3. Fecundities were normally distributed so parametric tests were justified. Only 3 lines had ≥ 5 females, so this criterion for line inclusion was relaxed

to ≥ 3 females. The importance of genotype on fecundity was determined in a one-way ANOVA of LINE effects. For subsequent correlations involving block 3, the median line fecundity was used rather than the mean so that the performance of lines in all blocks was measured on the same scale. It is noteworthy that because of the normality of the data, the mean and median of lines from block 3 were strongly and positively correlated ($r = 0.98$ $P < 0.001$).

Male and female longevity

Male and female longevity were analysed separately. The effect of line on longevity was investigated using two different estimates of longevity. Firstly, differences between lines in median longevity were identified using the median test (Zar 1996). Medians were calculable from all lines (except one) as > 50% of flies in each line had died by the end of the experiment. Dummy longevity values (equal to the largest true longevity value) were assigned to flies still alive at the end of the experiment to complete the data set. This procedure ensured that all flies were used for median tests. The median test is insensitive to the exact longevity values assigned to the surviving flies so long as they do not affect the grand median value. Second, the Kaplan-Meier survival functions of flies from each line were compared across lines using Log-rank tests. Survival was censored at 140 days post eclosion, as this was the duration between the time that the last fly was set-up and the end of the experiment. The estimated mean survival time generated by Kaplan-Meier analysis becomes biased when censored flies are present and underestimates the true mean survival time. The proportion of flies still alive in each line at the time of censorship was therefore employed as an alternate measure of longevity.

Correlations between male eyespan and components of fitness

To see if there was any genetic association between male eyespan expression and components of fitness correlations between the expression of the ornament in each environment and each component of fitness were assessed using Pearson's product-moment correlation coefficient (r). Qualitatively similar correlations were obtained using Spearman's rank correlation coefficient (r_s), but these data are not shown. Correlations were calculated using absolute measures of male eyespan and body size-controlled measures of male eyespan (eyespan LSMs). In order to establish whether life history traits showed any covariance with each other correlations between each component of fitness were computed.

Correlations of body size across generations

It was assumed that the genetic changes that took place over three consecutive generations of this experiment were small enough to allow the morphological, reproductive, and longevity estimates to be taken as characteristic of each line. This assumption was tested using correlations of body size across generations, the only trait that was common to each assay generation. A prerequisite for a significant genetic correlation is the existence of genetic variation in each of the variables. This was checked in one-way ANOVAs of LINE effects on thorax length (THX). Flies used in viability component assays were reared as larvae on *ad libitum* food, a similar level of developmental stress experienced by flies raised on ≥1.56 g corn per 13 eggs in the morphology assay. Thus correlations were computed using the least stressful treatment group only.

The influence of body size on components of viability

Body size differences between lines could result in correlated changes in components of viability if they show significant allometric relationships. Such "confounding" factors were evaluated by assessing the relationships between reproductive and longevity characteristics and body size. In the first instance, phenotypic associations were determined using correlations between the viability performance and body size of individual flies. Genetic correlations were then calculated as the correlation between line mean body size and estimates of line viability components. If any potential effect of body size scaling was detected then its importance was investigated in multiple regression-based GLMs of male eyespan (LSMs) and components of viability. In such instances, the mean line body size of flies used in the viability component assay(s) was included as a covariate (THX) and its significance was determined via an *F*-test on the change in explained variance upon removal of the THX term from the full model (Crawley 1993, p. 196).

Control Correlations Using Non-Sexual Traits

I have previously advocated a comparative approach to studying sexual signalling (Cotton *et al.* 2004a; Chapter 2). I therefore also computed correlations between components of fitness and non-sexual traits (female eyespan, and male and female wing length), both before and after correcting for body size. However, in order to minimise the number of statistical tests and associated type-I errors (see below), I only investigated non-sexual correlations with those components of viability that showed significant relationships with male eyespan.

6.3.7 Adjusting For Multiple Comparisons

In many cases multiple tests were performed on each dataset so the probability of making a type-I error was inflated. In such circumstances, adjustment of the significance level has been recommended (e.g. Rice 1989; Sokal & Rohlf 1995). However, the criteria for determining what constitutes a family of tests that needs to be analysed collectively are unclear (Rice 1989). For instance, it is uncertain whether correlations between a component of viability and male eyespan size from each of the three larval environments constitute three different tests, or a single comparison, especially given that line (least squares) mean trait sizes are not wholly independent of each other across the different treatments (Chapter 5). Therefore, rather than reporting amended significance levels for each test, unadjusted *P*-values are given and results of borderline significance are interpreted with caution.

6.4 RESULTS

6.4.1 Male Eyespan Condition Dependence

There was significant genetic variation (measured as the between-line component in an analysis of variance) in absolute male eyespan in all environments (> 0.12 g LINE $F_{21,387} = 6.76$, 0.03 g LINE $F_{21,407} = 7.14$, 0.015 g LINE $F_{17,294} = 5.74$, all $P < 0.001$). When thorax length (THX) was included in General Linear Models (GLMs) as a covariate estimate of body size, the significant differences between lines for

eyespan remained (with THX in the model: > 0.12 g LINE $F_{21,365} = 8.11$, 0.03 g LINE $F_{21,385} = 5.46$, 0.015 g LINE $F_{17,275} = 4.75$, all $P < 0.001$).

6.4.2 Male Fertility

There was no difference between lines for male fertility, as judged by the lack of significance of the LINE factor in a GLM with LINE and BLOCK effects (LINE $F_{18,88} = 1.38$, $P = 0.16$). The effect of the BLOCK was small, as % fertility LSMs were good estimates of line mean % fertility ($r = 0.95$, $P < 0.001$), and the same non-significant LINE factor was obtained in a one-way ANOVA of line effects on % fertility omitting the blocking variable (LINE $F_{18,90} = 1.33$, $P = 0.19$).

6.4.3 Female Fecundity

Results are given for the median fecundity of each line. However, qualitatively similar conclusions were obtained (but not presented here) using other metrics such as a) mean fecundity, b) mean fecundity of those laying eggs, and c), the proportion of females laying eggs.

Blocks 1 and 2

There were significant differences between lines for fecundity in the data set comprising blocks 1 and 2 ($\chi^2_{12} = 26.34$, $P = 0.009$). However, there was no evidence that this genetic variation in female reproductive output was signalled by male eyespan, as all correlations between median line fecundity and male eyespan were not significantly different from zero (Table 6.1).

Block 3

There was evidence for significant genetic variation between lines for fecundity in block 3 (LINE $F_{8,28} = 2.71$, $P = 0.024$). As with the data from blocks 1 and 2, there were no significant correlations between ornament expression and fecundity (Table 6.1).

6.4.5 Male And Female Longevity

Male longevity

Lines differed significantly for median male longevity ($\chi^2_{16} = 26.48$, $P = 0.048$). There was also significant heterogeneity of Kaplan-Meier male survival functions across lines (Log rank test: $\chi^2_{16} = 34.16$, $P = 0.005$), suggesting genetic variance in male longevity. The two estimates of male longevity were strongly and positively correlated ($r = 0.86$, $P < 0.001$). However, the average lifespan of a male genotype could not be predicted by his ornament size, as all correlations were non-significant (Table 6.2). A similar lack of association with male eyespan was found for the probability of surviving to 140 days post eclosion (Table 6.2).

Female longevity

Female median longevity was significantly influenced by female genotype ($\chi^2_{14} = 35.42$, $P = 0.001$), and female survival profiles also differed significantly between genotypes (Log rank test: $\chi^2_{14} = 34.30$, $P = 0.002$). Female median longevity and survival to 140 days were strongly and positively correlated across lines ($r = 0.92$, $P < 0.001$).

I found that there were consistent and significant correlations between estimates of female longevity and the expression of male eyespan after it was adjusted for body size (Table 6.3). Females from lines with a large male eyespan for their body size tended to be short-lived; genetic associations between male eyespan LSMs and female longevity were negative (Figure 6.1, Table 6.3).

6.4.6 Correlations Amongst Life History Traits

I found no evidence that any of the life history traits measured in this experiment were correlated with each other (Table 6.4).

6.4.7 Correlations Of Body Size Across Generations

There was significant variation between lines in body size in all assays (morphology > 0.12 g male LINE $F_{21,387} = 6.80$, female LINE $F_{21,365} = 7.41$, male fertility LINE $F_{18,90} = 2.96$, male longevity LINE $F_{16,186} = 6.09$, female fecundity LINE $F_{18,143} = 2.68$, female longevity LINE $F_{14,158} = 4.75$, all $P < 0.001$). There were also significant correlations of body size across generations (Table 6.5), suggesting that morphological and viability estimates of each line were affected little by any genetic changes that occurred over the three generations of the experiment. However, I did not find genetic similarity between male body size in the morphology assay and that of flies used to estimate male fertility. This implies that the sample of flies used in the male fertility assay was not representative of those used in the previous generation to assess morphology characteristics of each line.

6.4.8 The Influence Of Body Size On Components Of Viability

I found no phenotypic or genetic allometric relationships for any component of male viability or female fecundity (Table 6.6). I did find that larger females tended to live longer than smaller females (Table 6.6). However, there was no evidence for any significant genetic basis to this association as correlations between estimates of line longevity (measured as median longevity or survival to 140 days post eclosion) and line mean body size were not significantly different from zero (Table 6.6). Similarly, these findings had no effect on the correlations between male eyespan LSMs and female longevity, as inclusion of body size as a covariate in GLMs did not explain significantly more variation than models with just male eyespan LSMs (data not shown).

6.4.9 Control Correlations Using Non-Sexual Traits

Correlations between female longevity and non-sexual traits were undertaken as a control (after Cotton *et al.* 2004a; Chapter 2). There were no significant correlations between estimates of female longevity and any non-sexual trait (Table 6.7). However, and unlike male eyespan, female eyespan showed no significant associations with female longevity after correcting for body size (except one of marginal significance). Significant negative correlations between female longevity and body size-controlled male wing length were observed (Table 6.7).

6.5 DISCUSSION

"Good genes" models of sexual selection propose that costly female mate choice is adaptive if male ornaments signals heritable benefits that increase offspring fitness (Pomiankowski 1987a, 1988; Grafen 1990; Iwasa *et al.* 1991; Iwasa & Pomiankowski 1994; Houle & Kondrashov 2002). For analytical tractability, theoreticians have consolidated naturally selected components of fitness into a single "viability" proxy. But this is an over-simplification as heritable benefits of mate choice can arise through any of the (major) components of viability. Moreover, theory does not predict which aspects of fitness these will be, so one cannot make definitive *a priori* predictions about which components of viability will show genetic covariance with male ornament size. For example, patterns seen in one aspect of viability may simply be counterbalanced by opposing patterns in other, unmeasured, components. Whilst these problems can be overcome by measuring total fitness in the absence of sexual selection, the logistics of such experiments are typically prohibitive.

An often-overlooked element of "good genes" models is that the benefits of mate choice can be manifested in an increase in viability of female offspring, as well as male offspring. There have been relatively few studies that have explicitly investigated the genetic effects of male ornament size on the fitness of daughters (but see Promislow *et al.* 1998; Brooks 2000). In addition, if the negative genetic correlation between the sexes for adult fitness reported in *Drosophila* (Chippindale *et al.* 2001) is common in other species then total offspring viability cannot be reliably estimated from each sex in isolation. This leads to the requirement for "good genes"

studies to investigate genetic correlations between male ornament expression and numerous (major) components of viability *in each sex.*

I tested the expectation that male ornaments signals heritable benefits that increase offspring viability in the stalk-eyed fly, *C. dalmanni.* Males of this species possess exaggerated eyespan that shows heightened condition dependence relative to other traits (David *et al.* 1998; Cotton *et al.* 2004b, Chapter 3; Cotton *et al.* 2004c, Chapter 4). The potential for male eyespan to signal heritable properties is high as both its expression and its response to environmental stress shows significantly greater genetic variation than other traits (David *et al.* 2000; Chapter 5). Using correlations across genetically distinct inbred lines I assessed the relationships between male eyespan expression and the survival and reproductive components of male and female viability over three consecutive generations. Genetic changes that took place over this period were small enough to allow these properties to be taken as characteristic of each line for most traits. However, I did not find genetic similarity between flies used in the morphology assay and those used to estimate male fertility, so male fertility data should be regarded with suspicion. I also looked for any indication of antagonistic pleiotropy between the sexes for components of viability and whether there were any correlations between components of viability within each sex.

I found no evidence for any viability benefits associated with males bearing a large ornament. Male eyespan did not signal the genetic propensity of genotypes' fertility, fecundity or male longevity. However, after adjusting for body size, I found that male eyespan strongly and consistently predicted female longevity, although in the opposite direction to that predicted from the "good genes" model. Females from genotypes that produced a large male ornament for their body size lived significantly

shorter than females from genotypes that produced proportionately smaller ornaments.

Does the lack of positive correlations with fitness components mean that male eyespan signals only attractiveness benefits? To answer this question requires knowledge of the covariance between all major components of fitness and male ornament expression, as theory does not predict which components of viability will be signalled. This is clearly a difficult task, particularly since fitness has evolved in a natural arena that is almost impossible to replicate in the laboratory and many of the selective pressure present in the wild are notably absent (e.g. predation). The fitness traits as measured in this study (fertility, fecundity and longevity) may or may not be realistic estimates of those that are important in nature. For instance, although I found significant differences between the lines in both male and female longevity, survival was only measured from solitary individuals. *C. dalmanni* is partially gregarious, aggregating at night to roost and mate (Burkhardt & de la Motte 1985; Wilkinson & Dodson 1997), so any fitness benefits derived from asymmetric social interactions (e.g. Panhuis & Wilkinson 1999) will therefore have been undetectable in my study. In addition, it seems unlikely that there is no genetic variation in male fertility; if fertility had been measured or assayed differently then differences between the lines in male reproductive ability may have been uncovered. For instance, my measure of fertility was only made on single males in the absence of (sperm) competition with other rival males. I also only assayed adult fitness components, ignoring juvenile life history traits. Larval mortality is known to increase as larvae become stressed (Cotton *et al.* 2004b; Chapter 3) and early survival has potentially large effects on adult fitness (Williams 1966). Moreover, genes that provide benefits early in life may not always be good in later life (Stearns 1992), so positive larval viability effects may

out-weigh any genetically correlated reductions in adult viability. Assessment of differential larval survival with respect to genotype would therefore be a profitable start point for future study.

Just as environmental stress is known to have large effects on adult morphology in *C. dalmanni* (David *et al.* 1998, 2000; Cotton *et al.* 2004b, c; Chapters 3, 4, 5), it also has large detrimental effects on the expression of adult life history traits (Hingle *et al.* 2001b; Baker *et al.* 2003; S. Cotton *unpublished data*). As with ornament expression, one might expect the highest quality genotypes to be most resistant to stress and thus maintain high breeding values for components of fitness in all environments (*sensu* David *et al.* 2000; Chapter 5). The differences between genotypes in viability are also expected to increase as the environment deteriorates. Thus any covariance between male ornament expression and components of viability may only become biologically or statistically detectable under conditions of high stress. It is also noteworthy that episodes of high stress are precisely when any viability advantage will be most beneficial. So it remains unclear as to how, or if, the reaction norms of viability and male eyespan expression covary. Holometaboly creates additional complexity when trying to identify "good genes". Ornament size is fixed at eclosion so male eyespan can only signal adult viability attributes that are at least partially determined during larval or pupal development. All flies used in the viability assays were reared as larvae under benign conditions on *ad libitum* food, and were only stressed during the adult stage. I therefore have no information on the relationship between larval stress and adult viability, or on the general relationship (across a range of conditions) between components of viability and male eyespan expression.

It would be extremely informative to rear large numbers of larvae from each line under a range of *m* larval environments and then sub-divide each larval treatment group into further *n* treatment groups to be maintained under a gradient of environmental stress when adults. An experimental design of *n* adult stresses nested within *m* larval stresses would therefore allow investigation of correlations between male eyespan expression and the reaction norms of components of adult viability when adults had been either stressed as larvae or stressed as adults, or both. However, this would have required prodigious numbers of flies from each line, which I did not have. Most lines produced insufficient offspring each generation to distribute among more than one treatment, so for logistical reasons adult fitness components were only assayed in a single environment.

Contrary to the expectations of the handicap hypothesis, I found that male ornament expression was genetically associated with reductions in a component of female fitness. The same relationships were not found for the homologous female trait, suggesting that the utility of eyespan as a predictor of female longevity has evolved differently, or differs, between the sexes. I did find that male wing length covaried negatively with estimates of female longevity, but this was not unexpected given the qualitatively similar patterns of expression between male eyespan and male wing length (Chapter 5). However, note that many of the (male eyespan and wing) correlations were of borderline significance, and should be treated with caution. Such conservative advice would still have applied if correlations between eyespan and components of viability were positive, and thus been in support of the handicap hypothesis.

Rice (1992, 1996; see also Chippindale *et al.* 2001) has suggested that such phenomena are a consequence of sexually antagonistic genes, the result and cause of

perpetual co-evolution between males and females. Whilst there was no evidence for antagonistic pleiotropy within or between the sexes for viability components, I did find that genes that increase male fitness via attractiveness (i.e. those that produce a large ornament) do so at the detriment of female fitness (i.e. reduced female longevity) and *vice versa*. Thus females from lines that produced the largest male ornaments for their body size lived significantly shorter than females from genotypes that produced proportionately smaller ornaments.

Demonstrations of a genetic correlation between components of ornament expression and female longevity are not necessarily indicative of cause. All genetic models of sexual selection (whether handicap or Fisher) predict covariation between preference and display, and this has been reported on a number of occasions (in Diopsids, Wilkinson *et al.* 1998a, Wilkinson & Reillo 1994; see also Bakker 1993; Houde 1994; Blows 1999; reviewed in Bakker & Pomiankowski 1995; Bakker 1999). The costs of choice have typically been expressed as external, or environmentally determined (Pomiankowski 1987b), but it remains equally plausible that costly female choice could arise for genetic reasons. For instance, the reduced female longevity could be a pleiotropic effect of choice genes, and only genetically correlated with male eyespan via the linkage disequilibrium expected between preference and ornaments.

In conclusion, I found no evidence in *C. dalmanni* for genetic benefits of mate choice other than male attractiveness. The complexity of fitness means that the lack of genetic viability benefits for females who choose males with disproportionately large ornaments should not necessarily be taken as evidence against the "good genes" hypothesis. Future studies are necessary to examine in more detail the relationships between components of male and female viability, their interaction with the

environment and their genetic covariance with male ornament expression. I did find suggestive evidence that male ornaments do "tap-in" to the genetic processes that contribute to viability because the non-allometric component of male eyespan was negatively related to female longevity, suggesting that mate choice was costly for females. Again, additional work is required to assess the importance of this association in the evolution of exaggerated male eyespan and the female preferences for it.

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Table 6.1. Correlations between line estimates of female fecundity and male eyespan (ES) morphology. LSM = least squares mean estimate.

Variables		*n*	*r*
median fecundity (blocks 1 and 2)	ES (> 0.12 g)	13	0.02 $P = 0.96$
	ES (0.03 g)	13	0.26 $P = 0.39$
	ES (0.015 g)	10	0.46 $P = 0.18$
median fecundity (block 3)	ES (> 0.12 g)	9	-0.08 $P = 0.84$
	ES (0.03 g)	9	0.34 $P = 0.36$
	ES (0.015 g)	9	0.44 $P = 0.33$
median fecundity (blocks 1 and 2)	ES LSM (> 0.12 g)	13	0.03 $P = 0.93$
	ES LSM (0.03 g)	13	-0.11 $P = 0.73$
	ES LSM (0.015 g)	10	-0.05 $P = 0.90$
median fecundity (block 3)	ES LSM (> 0.12 g)	9	0.32 $P = 0.41$
	ES LSM (0.03 g)	9	0.24 $P = 0.54$
	ES LSM (0.015 g)	9	0.06 $P = 0.90$

Table 6.2. Correlations between line estimates of male longevity (measured as days (d) post eclosion) and male eyespan (ES) morphology. LSM = least squares mean estimate.

Variables		*n*	*r*
median longevity	ES (> 0.12 g)	16	0.12 $P = 0.66$
	ES (0.03 g)	16	-0.07 $P = 0.80$
	ES (0.015 g)	15	-0.03 $P = 0.90$
% survival at 140 d	ES (> 0.12 g)	16	0.05 $P = 0.85$
	ES (0.03 g)	16	0.09 $P = 0.73$
	ES (0.015 g)	15	0.16 $P = 0.58$
median longevity	ES LSM (> 0.12 g)	16	-0.13 $P = 0.64$
	ES LSM (0.03 g)	16	-0.02 $P = 0.95$
	ES LSM (0.015 g)	15	0.15 $P = 0.58$
% survival at 140 d	ES LSM (> 0.12 g)	16	-0.11 $P = 0.69$
	ES LSM (0.03 g)	16	0.15 $P = 0.59$
	ES LSM (0.015 g)	15	0.15 $P = 0.60$

Table 6.3. Correlations between line estimates of female longevity (measured as days (d) post eclosion) and male eyespan (ES) morphology. LSM = least squares mean estimate. Significant correlations are in bold-type.

Variables		*n*	*r*
median longevity	ES (> 0.12 g)	15	-0.24 $P = 0.38$
	ES (0.03 g)	15	0.002 $P = 0.99$
	ES (0.015 g)	14	-0.11 $P = 0.71$
% survival at 140 d	ES (> 0.12 g)	15	-0.24 $P = 0.36$
	ES (0.03 g)	15	0.02 $P = 0.95$
	ES (0.015 g)	14	-0.06 $P = 0.83$
median longevity	ES LSM (> 0.12 g)	15	**-0.67 $P = 0.006$**
	ES LSM (0.03 g)	15	**-0.51 $P = 0.049$**
	ES LSM (0.015 g)	14	**-0.62 $P = 0.017$**
% survival at 140 d	ES LSM (> 0.12 g)	15	**-0.61 $P = 0.011$**
	ES LSM (0.03 g)	15	-0.42 $P = 0.11$
	ES LSM (0.015 g)	14	**-0.54 $P = 0.039$**

Table 6.4. Correlations between male and female life history traits. Correlations involving male fertility are not included because there was no significant difference between the lines in this measure of fitness.

Variables		*n*	*r*
female fecundity (blocks 1 & 2)	female median longevity	10	0.51 $P = 0.14$
female fecundity (block 3)	female median longevity	7	0.14 $P = 0.77$
female fecundity (blocks 1 & 2)	female % survival at 140 d	11	0.36 $P = 0.27$
female fecundity (block 3)	female % survival at 140 d	7	-0.01 $P = 0.99$
female fecundity (blocks 1 & 2)	male median longevity	11	-0.07 $P = 0.85$
female fecundity (block 3)	male median longevity	7	0.17 $P = 0.72$
female fecundity (blocks 1 & 2)	male % survival at 140 d	11	-0.16 $P = 0.63$
female fecundity (block 3)	male % survival at 140 d	7	0.61 $P = 0.15$
male median longevity	female median longevity	15	0.26 $P = 0.35$
male % survival at 140 d	female % survival at 140 d	16	0.22 $P = 0.42$

Table 6.5. Correlations of thorax length (THX) in the > 0.12 g treatment of the morphology (morph.) assay and thorax length from the viability assays (fert. = fertility, fec. = fecundity, long. = longevity). Significant correlations are in bold-type.

Variables		*n*	*r*
male morph. THX	male fert. THX	18	-0.02 $P = 0.93$
male morph. THX	male long. THX	16	**0.57 $P = 0.02$**
female morph. THX	female fec. THX	18	**0.65 $P = 0.003$**
female morph. THX	female long. THX	14	0.51 $P = 0.06$

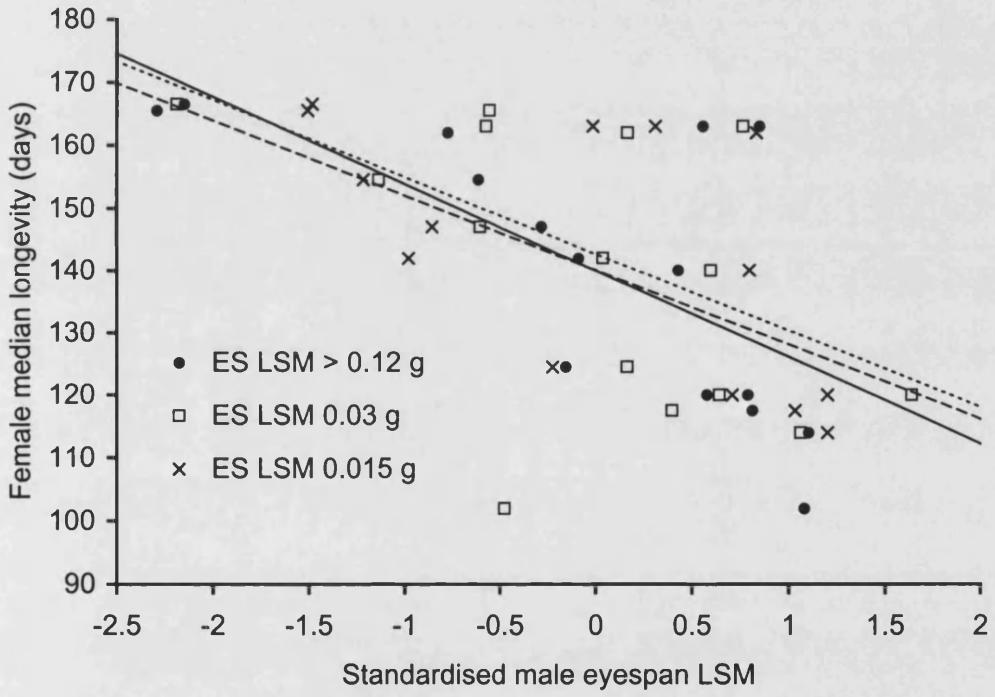
Table 6.6. Phenotypic (r_P) and genetic (r_G) correlations between components of viability and body size (thorax length) in the viability assays (THX = thorax length, fert. = fertility, med. long. = median longevity, surv. to 140 d = survival to 140 days post eclosion, fec. = fecundity (numbers in parentheses indicate block numbers)). † = phenotypic correlation between censor value in survival analysis (= 0 or 1) and body size. Significant correlations are in bold-type.

Variables		r_P	n	r_G	n
male % fert.	male % fert. THX	-0.006	109	0.10	18
		$P = 0.95$		$P = 0.68$	
male med. long.	male long. THX	0.03	202	0.22	17
		$P = 0.63$		$P = 0.40$	
male % surv. to 140 d	male long. THX	0.06†	202	0.12	17
		$P = 0.38$		$P = 0.66$	
female fec. (1&2)	female fec. THX	0.03	121	0.24	13
		$P = 0.75$		$P = 0.43$	
female fec. (3)	female fec. THX	-0.03	39	0.54	9
		$P = 0.88$		$P = 0.14$	
female med. long.	female long. THX	**0.22**	173	0.40	15
		$P = 0.004$		$P = 0.14$	
female % surv. to 140 d	female long. THX	**0.24†**	173	0.41	15
		$P = 0.002$		$P = 0.13$	

Table 6.7. Correlations between line estimates of female longevity (measured as days (d) post eclosion) and line mean non-sexual trait morphology. ES = eyespan, wing = wing length. LSM = least squares mean estimate. Numbers in parentheses indicate sample size of correlation). Significant correlations are in bold-type.

Variables		*r* (male wing)	*r* (female ES)	*r* (female wing)
median longevity	mean (> 0.12 g)	-0.38 $P = 0.17$ (15)	-0.21 $P = 0.46$ (14)	-0.16 $P = 0.58$ (14)
	mean (0.03 g)	-0.09 $P = 0.75$ (15)	0.003 $P = 0.99$ (15)	0.07 $P = 0.82$ (15)
	mean (0.015 g)	-0.19 $P = 0.51$ (14)	-0.15 $P = 0.58$ (15)	-0.14 $P = 0.61$ (15)
% survival at 140 d	mean (> 0.12 g)	-0.38 $P = 0.15$ (16)	-0.14 $P = 0.61$ (15)	-0.06 $P = 0.85$ (15)
	mean (0.03 g)	-0.04 $P = 0.90$ (16)	-0.15 $P = 0.57$ (16)	-0.01 $P = 0.96$ (16)
	mean (0.015 g)	-0.14 $P = 0.63$ (15)	-0.18 $P = 0.50$ (16)	-0.16 $P = 0.56$ (16)
median longevity	LSM (> 0.12 g)	**-0.63 $P = 0.012$** (15)	-0.42 $P = 0.13$ (14)	-0.25 $P = 0.40$ (14)
	LSM (0.03 g)	**-0.54 $P = 0.036$** (15)	-0.34 $P = 0.21$ (15)	0.001 $P = 0.99$ (15)
	LSM (0.015 g)	**-0.64 $P = 0.013$** (14)	-0.35 $P = 0.21$ (15)	-0.21 $P = 0.44$ (15)
% survival at 140 d	LSM (> 0.12 g)	**-0.60 $P = 0.014$** (16)	-0.39 $P = 0.15$ (15)	-0.21 $P = 0.46$ (15)
	LSM (0.03 g)	-0.41 $P = 0.11$ (16)	**-0.51 $P = 0.046$** (16)	-0.05 $P = 0.85$ (16)
	LSM (0.015 g)	**-0.55 $P = 0.035$** (15)	-0.47 $P = 0.07$ (16)	-0.26 $P = 0.34$ (16)

Figure 6.1. Correlations between female median longevity and male eyespan (ES) least squares mean (LSM) estimates from each of the three food treatments (> 0.12 g, 0.03 g and 0.015 g per egg). Male eyespan LSMs are displayed as standardised normal deviates (= $(x - \bar{x})/SD_x$) to ease comparisons between treatments. See Table 6.3 for correlation coefficients and significance values. Least squares regression lines are fitted for heuristic purposes: solid line = > 0.12 g treatment, dashed line = 0.03 g treatment, dotted line = 0.015 g treatment.



7

General Discussion

7.1 RECAPITULATION AND SUMMARY OF FINDINGS

In many species, females prefer to mate with males sporting the most elaborate sexual ornaments (Andersson 1994). The handicap model of sexual selection proposes that ornaments signal male genetic quality, so females mating with the most ornamented males acquire paternally derived fitness benefits for their offspring (Andersson 1986; Pomiankowski 1987, 1988; Grafen 1990; Iwasa *et al.* 1991; Iwasa & Pomiankowski 1994). Male ornaments are predicted to have co-evolved with female preference to be larger, and so more costly. This leads to the expectation that ornament expression should be highly sensitive to environmental and genetic conditions. The widespread occurrence of male sexual ornaments has lead to eugenic sexual selection, and its emergent properties, becoming one of the most hotly contested topics in evolutionary biology.

The core theme running through this thesis was the hypothesis that male sexual ornaments have evolved heightened condition dependence. The dissertation began by providing a synthesis of the theoretical resolutions that have been proposed to account for male ornaments, and female preferences for them (Chapter 1). This was followed by a critical re-assessment of the widely held view that male sexual ornaments are condition-dependent (Chapter 2). I then proceeded to experimentally investigate the condition dependence (Chapters 3,4), genetics (Chapter 5), and covariation with components of fitness (Chapter 6), of male eyespan in stalk-eyed flies, in an effort to elucidate the signalling function of exaggerated male ornaments in Diopsids. In the following section I briefly summarise the findings of each chapter.

Chapter 1: General Introduction

Natural selection cannot explain the obviously maladaptive secondary sexual characteristics of males from many species. Darwin (1859, 1871) suggested that ornaments are beneficial because males bearing such characteristics are often preferred as mates by females. Fisher (1915, 1930) proposed that female preferences would originally evolve for traits that confer a natural selection advantage. However, as a result of non-random mating, female preference alleles become linked to alleles for the preferred trait and may initiate a "runaway process" between male traits and preference genes that causes exaggeration of male ornaments. In contrast, Zahavi (1975, 1977) argued that ornaments acted as a "handicap" and evolved precisely because they were costly. Only males of high viability can survive with the burden of a large deleterious ornamental trait, so females exhibiting preference for handicapped males will have offspring of higher than average viability and so be favoured by selection. The handicap principle works provided two criteria are met, 1) that there is sufficient genetic variation in fitness to permit positive parent-on-offspring correlations of viability, and 2), that male ornaments are expressed in proportion to the condition of their bearer. The former condition is readily met, but evidence for the latter is often unclear (see Chapter 2).

Chapter 2: Do Sexual Ornaments Demonstrate Heightened Condition-Dependent Expression As Predicted By The Handicap Hypothesis?

A key prediction made by the handicap hypothesis is that male sexual traits have evolved heightened condition-dependent expression, as a result of the higher differential cost of ornaments relative to other non-ornamental traits (Iwasa & Pomiankowski 1994). The prediction has only recently been subject to experimental investigation. I reviewed published experimental data and found that there are few well-designed tests that support this critical hypothesis. Most studies have overlooked the comparative aspect of this hypothesis and have neglected to contrast the condition dependence of ornaments with that of suitable non-sexual traits. Few experiments have adequately accounted for the influence of body size scaling on sexual trait expression, and most have failed to assay individuals under a range of stresses representative of those experienced in nature. Finally, there is a dearth of experimental studies exploring the genetic basis of condition dependence, a feature that is crucial for the handicap hypothesis. The key, and unexpected, finding from my review is that there is little support from well-designed experiments for the common claim that sexual ornaments show heightened condition dependence. This chapter included recommendations of the appropriate requirements for rigorous studies of condition dependence. These recommendations were followed in subsequent chapters of my thesis.

Chapter 3: Condition Dependence Of Sexual Ornament Size And Variation In The Stalk-Eyed Fly *Cyrtodiopsis dalmanni* (Diptera: Diopsidae)

I used the stalk-eyed fly *Cyrtodiopsis dalmanni* to examine predictions made by condition-dependent handicap models of sexual selection. *C. dalmanni* is highly

sexually dimorphic for eyespan, and females exhibit strong mating preferences for males with large eyespans, both before and after controlling for body size. Condition was experimentally varied by manipulation of larval food availability. I found that male eyespan was more sensitive to changes in condition than the homologous character in females and other non-sexual traits. Male eyespan also showed a great increase in standardized variance under stress, unlike non-sexual traits. These patterns persisted both before and after controlling for body size, suggesting that females can gain additional information about male phenotypic condition by assessment of eyespan over and above any effects of other traits or body size. These findings accord well with condition-dependent handicap models of sexual selection.

Chapter 4: Heightened Condition Dependence Is Not A General Feature Of Male Eyespan In Stalk-Eyed Flies (Diptera: Diopsidae)

The experimental regime used in Chapter 3 was applied to *Sphyracephala beccarri*. Male eyespan is unexaggerated in *S. beccarri* and there is no evidence for female mate choice in this species. I used *S. beccarri* to test the hypothesis that heightened condition dependence evolves only when ornaments are subject to strong sexual selection and become costly through exaggeration. Against this prediction, I found that male eyespan was more sensitive to changes in condition than a control trait in males (wing length), using both absolute and body size-controlled measurements. However, female eyespan showed a similar pattern of greater sensitivity, and there was no difference between the sexes in the degree of increased eyespan sensitivity when compared to wing length. I also compared the response to stress in *S. beccarri* with that in *C. dalmanni*, to demonstrate the positive association

of heightened condition dependence with traits that have become exaggerated through sexual selection. The finding in *S. beccarri* that eyespan is naturally more sensitive to condition than other traits, and that this may have acted as a pre-adaptation to its role in sexual signalling in other Diopsid species. These results are consistent with handicap models of sexual selection, and also support Fisher's (1915, 1930) original view of how sexual selection is initiated.

Chapter 5: The Genetic Basis Of Heightened Condition Dependence In The Stalk-Eyed Fly *Cyrtodiopsis dalmanni* (Diptera: Diopsidae)

Genetic models of the handicap principle assume a heritable basis to condition-dependent ornament expression. I extended my findings from Chapter 3 and tested this expectation in *C. dalmanni*. I created distinct genotypes using inbreeding, and the genetic basis of ornament condition dependence was investigated by comparing the performance of genotypes (inbred lines) along a gradient of environmental stress. I found significant interactions between genotype and environmental stress for male eyespan; lines that produced a large ornament in one environment tended to do so in others. Importantly, stress amplified these differences between genotypes leading to an increase in the genetic variance of the male ornament. Such patterns were present to a much lesser extent in non-sexual traits, and persisted after controlling for size. These results are predicted by handicap models of sexual selection and suggest that "good genes" are important in the evolution of exaggerated male eyespan in this species and the female preferences for it.

Chapter 6: Does Male Eyespan Covary Genetically With Components Of Fitness In The Stalk-Eyed Fly *Cyrtodiopsis dalmanni* (Diptera: Diopsidae)?

The morphological findings of Chapter 5 were used to examine the genetic correlations between male eyespan expression and four components of fitness (male fertility, female fecundity, and male and female longevity). This allowed me to test the hypothesis that male eyespan is positively correlated with viability, and that females can obtain genetic benefits for their offspring by mating with well-ornamented males. I found that male eyespan expression did not predict either component of male fitness or female fecundity. However, after adjusting for body size, I found that male eyespan was negatively correlated with estimates of female longevity. These findings were unexpected and do not provide support for "good genes" benefits of sexual selection.

To summarise, my thesis evaluates the under-explored assumption that ornaments have evolved heightened condition dependence. It provides a framework within which condition dependence can be rigorously tested. I used this experimental scaffold to evaluate the condition dependence of male eyespan in stalk-eyed flies and found that, as predicted, male ornaments showed elevated sensitivity to environmental and genetic conditions. Nonetheless, I failed to find any positive correlations between ornament expression and components of fitness (divorced from attractiveness). Thus the Fisherian benefit of attractive sons was the only genetic advantage for female preference that I found. However, the findings of Chapter 6 should only be taken as

preliminary and a fuller examination of the covariance between male eyespan and fitness is required (see below).

7.2 FUTURE DIRECTIONS

Whilst condition dependence has been extensively investigated here as both a general phenomenon and as a characteristic within stalk-eyed flies, this is by no means an exhaustive treatise. Below I identify four broad areas, with reference to my work on stalk-eyed flies, which would benefit from future study.

7.2.1 Phylogenetic Patterns Of Condition Dependence

Iwasa and Pomiankowski (1994) showed that the degree of condition dependence is proportional to the differential cost of an ornament (reviewed in Cotton *et al.* 2004a; Chapters 1, 2). Thus as ornaments become more exaggerated, and so more costly, they become more dependent on male condition. If the cost differential increases in proportion with the degree of ornament exaggeration across species, then species with little or no ornament exaggeration should exhibit little or no heightened condition dependence. By contrast, species with highly exaggerated sexual ornaments should show greatly elevated condition dependence. This comparative question is ideally suited to a phylogenetic analysis. However, as a result of the quantitative, rather than qualitative, nature of condition dependence, tests should be designed to detect an evolved change in the degree of ornament condition dependence rather than its existence *per se*.

The phylogeny of the Diopsidae is well characterised (Baker *et al.* 2001) and the extensive changes, both gains and losses, of eyespan exaggeration, and the concurrent changes in female mate preferences (Wilkinson *et al.* 1998; Baker & Wilkinson 2001) make this clade ideal for a comparative treatment of sexual ornament condition dependence. An initial phylogenetic analysis by Wilkinson and Taper (1999) suggests that condition dependence is indeed associated with eyespan exaggeration. However, their approach is correlational (body size was used as an index of condition) and overlooks the significant non-allometric component of eyespan condition dependence (David *et al.* 1998; Cotton *et al.* 2004b, c; Chapters 3, 4, 5 (see also chapter 6)). Experimental investigation of condition dependence would overcome these problems. I have already shown that *C. dalmanni*, a species with highly exaggerated eyespan, has greatly elevated condition dependence (Cotton *et al.* 2004b; Chapters 3, 5), whereas the unexaggerated male eyespan of *S. beccarri* does not exhibit heightened condition dependence (Cotton *et al.* 2004c, Chapter 4). However, this contrast uses species at the extremes of eyespan exaggeration, so it remains unclear how the eyespan of intermediates behaves. Furthermore, *C. dalmanni* and *S. beccarri* are phylogenetically distant (Baker *et al.* 2001; Baker & Wilkinson 2001), so examination of more species is required to test the generality of this result. For instance, the *Cyrtodiopsis*/ *Teleopsis* branch captures the complete range of eyespan diversity, ranging from monomorphism (*C. quinquegutatta* and *T. quadriguttata*) through moderate (*T. rubicunda*) and high sexual dimorphism (*C. dalmanni* and *C. whitei*) to extreme sex differences in eyespan (*T. breviscopium*) (Baker & Wilkinson 2001). Extension of the findings of Chapter 4 (Cotton *et al.* 2004c) are also possible, as the *Sphyracephela* genus contains species such as *S. bipunctipennis* which has evolved marked sexual dimorphism for eyespan, and *S.*

brevicornis which has evolved sexual monomorphism for eyespan allometry as well as for absolute eyespan (Baker & Wilkinson 2001).

7.2.2 Direct Manipulation Of Genetic Variation

All experimental studies assessing genetic variation in condition dependence have exploited standing genetic variation in their focal population(s) using full- and/or half-sib designs (David *et al.* 2000; Kotiaho *et al.* 2001), artificial selection (Jia *et al.* 2000) or inbreeding (Sheridan & Pomainkowski 1997; Van Oosterhout *et al.* 2003; Chapter 5) (reviewed in Cotton *et al.* 2004a; Chapter 2). Thus they are all partially correlative. To date, direct manipulation of genetic quality and evaluation of the subsequent changes in ornament size (relative to control traits) has not been attempted. A possible experiment was outlined in Chapter 2 (Cotton *et al.* 2004a). If mutation load was manipulated then one would expect groups with the most mutations to have smaller ornaments than controls reared in the same environment; non-sexual trait expression would be expected to differ much less between treatments. In addition, if (components of) fitness of the elevated mutation group were also reduced relative to controls, then such pleiotropy would provide strong evidence that ornaments are sensitive to, and signal, the mutation load of their bearer. These predictions are based on the assumption that mutations are biased, which seems likely (Iwasa *et al.* 1991; Pomiankowski *et al.* 1991). One possible method for manipulating mutation load would be the application of a mutagen, such as ethyl methanesulphonate (EMS) or triethylenemelamine (TEM), which induces point mutations or small deletions throughout the genome (Grigliatti 1986).

Such an experiment would be most suited for species where there is an *a priori* expectation that ornaments are condition-dependent and where the required large sample sizes are easily obtainable. Stalk-eyed flies, and *C. dalmanni* in particular, would be ideal candidates as there is strong reason to believe that exaggerated male eyespan is condition-dependent (David *et al.* 1998, 2000; Cotton *et al.* 2004b; Chapters 3, 5), and they can be cultured in large numbers with relative ease in the laboratory.

7.2.3 Covariation Between Ornament Expression And Fitness

One of the principle predictions of the handicap theory is that the genetic variance in ornament size will increase with environmental stress, and that the relative performance of genotypes, with respect to ornament size, will to be maintained across environments (Chapters 2, 5). In short, genotypes that produce a large ornament in one environment should also do so in others. However, as a result of lower differential costs of ornament expression, genetic correlations of ornament size are expected to weaken as environments become more benign. Similarly, the highest quality genotypes are expected to maintain high breeding values for components of fitness in all environments, so differences between genotypes in viability are also expected to increase as the environment deteriorates. Thus investigations that fail to find positive genetic correlations between ornament size and (components of) fitness may be assaying animals under experimental conditions that are not stressful enough to reveal any such correlations. It would therefore be profitable to investigate the reaction norms of (components of) fitness or viability as well as those of ornament expression, and look for covariation between the two types of reaction norm. Such

experiments should be duplicated using viability measures from both larvae and adult flies propagated under a gradient of environmental stress. *A priori,* correlations are expected to be strongest under the most severe environments. An approach similar to that just described is the next logical step for continuing the findings (or lack thereof) reported in Chapter 6.

7.2.4 Relating Laboratory Findings To Behaviour And Development In The Field

Throughout this thesis I have emphasized the need for controlled experiments. This usually necessitates laboratory-based investigations using captive animals. Since selective pressures acting on natural and domesticated populations are likely to be different, then conclusions drawn from studies on captive populations may not hold in the natural arena. A combination of laboratory and field studies would therefore provide a more realistic picture of condition-dependent ornament expression. By experimentally investigating the response to environmental stress in the field, one can make biologically realistic comparisons that are free from the drawbacks of using laboratory-adapted subjects. Experimental field manipulations have typically been used in studies of avian ornament condition dependence (e.g. Gustaffson *et al.* 1995; Qvarnström 1999; Griffith 2000), but none of these have comparable laboratory homologues. In the cricket *Gryllus campestris* however, condition dependence of calling song has been investigated in both controlled laboratory experiments (Scheuber *et al.* 2003) and field manipulations (Holzer *et al.* 2003), with both types of study producing similar results.

To date, no manipulative field experiments have been performed on the Diopsidae and few data are available on the natural phenotypic or genetic variance of morphological traits (but see Burkhardt & de la Motte 1985). Since the phenotypic and genetic variances in male *C. dalmanni* eyespan are negatively correlated with the quality of the environment (David *et al.* 2000; Cotton *et al.* 2004b; Chapters 3, 5), then a possible experiment would be to augment oviposition/larval development sites with food in one or more geographically isolated populations, and contrast size and variation in male eyespan with that of other non-sexual traits between manipulated and control populations. An alternative, and less experimental approach, would be to quantify a number of non-continuous habitat types in terms of an index describing the prevalence and abundance of decaying material within the area (i.e. a measure of the quantity and quality of larval development sites), and look for the expected negative (and positive) correlations between habitat quality and the mean size (and variance) of male eyespan.

To conclude, it will be important to verify the generality of my findings by examining a diverse array of other stalk-eyed fly species. Within *C. dalmanni*, my findings on the genetics of condition dependence and the covariation of male eyespan with fitness need to be extended by using direct manipulation of fitness (mutation load), and by integrating the effects of environmental stress on such covariation. This work ought to be verified under natural environments. Only then can we begin to understand fully the signalling function of exaggerated male eyespan in stalk-eyed flies, and of sexual ornaments in general.

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